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THE RELATION OF THE SEED-CORN MAGGOT (PHORBIA FUSCICEPS ZETT.) TO THE SPREAD AND DEVELOPMENT OF POTATO BLACKLEG IN MINNESOTA[1]

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INTRODUCTION

It has been assumed generally by plant pathologists that the pathogene of potato blackleg overwinters in, and is spread only by, infected tubers. Morse (22), in 1917, stated that "Observations in Maine indicate that under the climatic conditions which exist there infected seed potatoes are the sole source of infection and distribution . . ." The only other possible source of infection that has received much attention is the soil.

Rosenbaum and Ramsey (27) could find no evidence to indicate that the pathogene could survive in the soil, or in diseased tubers in the soil, in Aroostook County, Maine, or at Norfolk, Virginia. They placed both naturally and artificially infected tubers in the soil in the fall, and planted selected and disinfected tubers the following spring, in the rows where the diseased tubers had been planted the previous fall. No blackleg developed, and they were unable to reisolate the pathogene from the soil.

Ramsey (25) inoculated one-half-pint jars of soil with 15 cc. of a broth culture of the blackleg pathogene and buried them in the soil over winter, but he was unable to reisolate the organism by dilution cultures the following spring. He also watered growing potato plants heavily with broth cultures of the pathogene. In other cases the soil was inoculated by incorporating decayed tubers, some of which were placed in direct contact with the seed piece. Only one plant of the many inoculated developed the disease. From these experiments it was concluded that "Unless the seed piece is infected at planting time, there is little chance that uninjured plants will contract the disease even though the causal organism is washed about the stem and root system."

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Pethybridge (24) in Ireland, and Murphy (23) in Canada, held similar views.

On the other hand, von Hegyi (14), working in Polish Galicia and Prussian Silesia, found such a close correlation between blackleg and wire worm attack that he held biting insects to be a necessary factor for entry of the parasite. Little attention has been given by subsequent workers to this insect or other insects as possible means of spread of the disease.

Jennison (16) states that "The larvae of insects have been found by the writer working in and on the affected tissues, but there was no positive evidence that they were active agents in the dispersal of the disease." No experiments with the insects were mentioned.

Kotila and Coons (18) showed that in Michigan under certain conditions the bacteria overwintered in tubers remaining in the soil. They also showed that the blackleg pathogene was present in the soil surrounding diseased plants, but concluded that "It does not, however, seem capable of existing there for any considerable length of time."

Murphy (23) states the prevailing attitude of pathologists toward the question as follows:

"Although there seems to be considerable reluctance in finally accepting the view that this disease is not, for practical purposes, harbored or spread by the soil, failure to bring about infection from this source, and the success of control measures applied to the tubers, leave no other alternative open. The skepticism which has prevailed as to the absence of soil infection is to be traced to three, or probably four, factors: the failure to account for certain widespread outbreaks in plants produced from seed tubers presumed to be practically uninfected; the want of any entirely satisfactory theory of hibernation in the tubers; the failure of attacks to develop in certain cases from infected seed potatoes under conditions presumed favorable; and the possible confusing of fusarium-wilt with blackleg."

Since the summer of 1920 the writer has had occasion to investigate numerous outbreaks of blackleg in Minnesota which could not be satisfactorily explained on the basis of our present knowledge. Many of the fields investigated had been planted with certified seed from fields known to be free from blackleg, and the seed had been properly disinfected and planted with the best of care. It was thought at first that the disease might have been spread from a few infected tubers in the cutting operations, for Morse (21) states that "There is every reason to believe that in cutting seed the disease may be spread from diseased to healthy seed pieces by contact of their freshly cut, moist surfaces and less frequently from the hands of the operator or through the medium of the knives used." However, he cites no experiments to support the statement.

ATTEMPTS TO PRODUCE THE DISEASE BY SEED PIECE INOCULATION

In the spring of 1922, experiments were planned to determine the possibility of spread of the disease by seed piece contamination during the cutting operation. A quantity of tubers were inoculated with a virulent culture of the blackleg pathogene which previously had been tested by inoculating stems of growing plants in the greenhouse. After incubation in a moist chamber for several days, the tubers were completely decayed. The cut surfaces of sixty freshly cut seed pieces were thoroughly smeared with the rotted tubers. These in turn were incubated for twenty-four hours in a moist chamber and then planted at University Farm. Much to the surprise of the writer, not a single case of blackleg developed.

The following year this experiment was repeated on a larger scale, using a more positive means of inoculation. About four hundred freshly cut

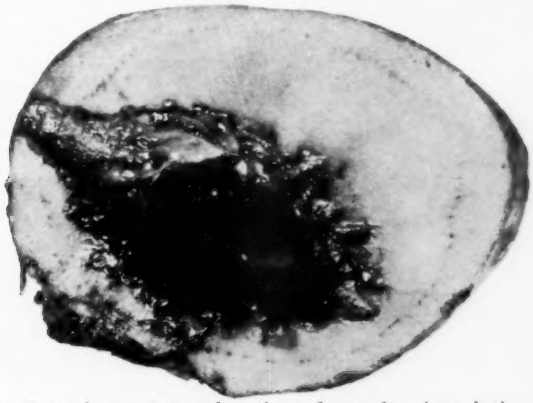

FIG. 1. A section through a potato tuber three days after inoculation with the blackleg pathogene by the method described in the text: typical of tubers used in the experiments on seed piece inoculation.

seed pieces were placed in large crocks, sprayed with a broth culture of the pathogene,[2] and incubated until the decay had extended to an average depth of approximately one-fourth inch. Some of the seed pieces were punctured through the skin and decay was also started at these points. Some of the tubers were planted on mineral soil, and some on peat soil, where a variation in the water level from one to five feet was maintained. Again not a single case of blackleg developed, although a few of the badly rotted seed pieces decayed completely before a sprout could be sent out.

[2] In this and all subsequent inoculation experiments, two cultures of the pathogene were used. One was isolated and tested by the writer, and the second was received from Dr. Erwin F. Smith under the name "*Bacillus phytophthorus* Appel I 1906." Both cultures were strongly pathogenic to potato stems and tubers.

Many of the seed pieces were later dug up and examined, and, in every case, it was found that the decay had been effectively walled off with a thick layer of cork cells. These results would indicate strongly that the disease was not commonly spread in this way. However, it was thought that, since the decay extended from the surface inward and was in direct contact with the soil, some detrimental action of the soil might be responsible for the failure of the pathogene to develop. Accordingly, the experiment was repeated in 1924, using a different method of inoculation. A hole about one inch deep was bored into the tuber with a one-fourth-inch wood

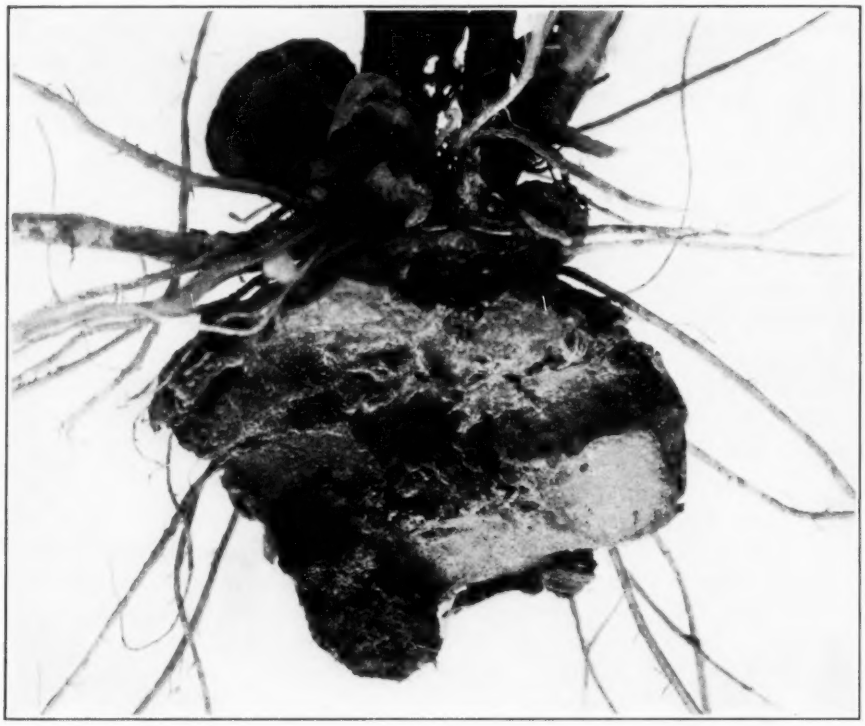

FIG. 2. A seed piece, which had been inoculated with the blackleg pathogene, dug up more than two months after planting in the field. The decayed portion had been walled off entirely by a layer of cork. The outline of the decayed cavity and the inoculation hole are still visible.

bit. The hole was almost filled with a broth culture of the pathogene and then plugged with a tightly fitting plug cut with a cork borer from another tuber. After incubation for three days, the decay was found to be developing rapidly (Fig. 1). The tubers were planted without disturbing the plug. The results were the same as in previous years. Not a single case

of blackleg developed, and, on examination, it was seen that the seed piece had effectively corked off the decay (Figs. 2 and 3, Plate XV, l, m, and n). These experiments seem to indicate conclusively that seed piece infection at cutting time is not an important factor in the development of the disease, providing the seed piece is planted under conditions not inhibitory to cork formation.

THE ASSOCIATION OF THE SEED-CORN MAGGOT WITH BLACKLEG PLANTS

In the summer of 1923, while seeking further for an explanation of the outbreaks of blackleg from seed apparently free from the disease, a large number of diseased plants were dug up, seed piece and all, and carefully examined. Most of the observations were made fairly late in the season, after the disease was well advanced. In many plants a number of small white maggots were found working in the stems. In a few cases the maggots were found in the old seed pieces, but in most cases only fragments of the seed piece were left. It was impossible at this time to tell whether the maggots were of any significance or whether they were merely feeding on the plants which had already started to decay. However, a number of the maggots were collected and reared to maturity. Several different

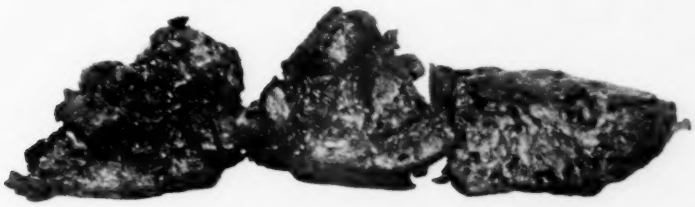

FIG. 3. Seed pieces infested with seed-corn maggots. These seed pieces had been planted about thirty days, and the plants were just beginning to show typical symptoms of blackleg. Note the slimy burrows. The maggots are practically full grown and almost ready to enter the soil and pupate.

species were obtained, but most prevalent was the seed-corn maggot (*Phorbia fusciceps* Zett.).[3] Inasmuch as this fly is recognized as a parasitic insect on a number of crop plants, it appeared that its presence in potato plants affected with blackleg might be more than incidental.

In the spring of 1924 a very thorough study was made of the relation of the insect to the development of blackleg. Unfortunately, on account

[3] These first specimens were kindly identified by Dr. J. R. Eyer, formerly assistant in entomology at the University of Minnesota. Later specimens were sent to Dr. J. M. Aldrich of the National Museum and to Dr. O. A. Johannsen of Cornell University, both of whom verified the identification. The insect is probably more properly called *Hylemyia cilicrura* Rond, but, since it is better known in American literature on economic entomology as *Phorbia fusciceps*, this name has been used.

of other duties, the writer was unable to make any extensive observations at the time of planting. On June 26, shortly after the potato plants had appeared above ground in the Red River Valley, a field laboratory was established at the Northwest School of Agriculture at Crookston, Minnesota. The first infected plants were observed on June 29 in a field of Early Ohio potatoes, in which the healthy plants were about six inches tall. About one per cent of the plants were beginning to show the characteristic symptoms of the disease. In the next three days more than a hundred diseased plants were dug up and examined. Maggots were found in the seed pieces of all but six plants (Fig. 3). The maggots varied in length from four to seven mm., and some were beginning to pupate. A puparium was found in the soil near one of the six plants. The other five seed pieces yielded no maggots, although it is possible that puparia may have been overlooked. Two days later, when a more careful examination of the surrounding soil was being made, several puparia and a few pupal skins were found.

During the next three weeks more than 1,000 plants were examined. The plants came from about fifty different fields in three counties. Each successive day the number of puparia in proportion to maggots increased. Pupation was at its height by July 4, and many adults were emerging, as indicated by the pupal cases found.

Several hundred maggots and puparia were collected and put in bottles of soil which were placed in a screen cage in the laboratory. Practically all of these eventually developed into adult flies. Of more than three hundred flies obtained in this way, all but seven were identified as the seed-corn maggot fly.

THE SEED-CORN MAGGOT

The seed-corn maggot, which has been somewhat inappropriately named, is closely related to the better-known cabbage maggot (*Phorbia brassicae* Bouche). The adult insect is a fly slightly smaller than the common house fly (Fig. 8). The larvae are known to be parasitic on a number of crop plants. In a brief survey of the literature, reports of injury on corn, beans, peas, turnips, cabbage, radishes, onions, beets, potatoes, hedge mustard (8), tomatoes (30), and wheat (28) were found. In New Jersey the insect is considered a serious pest of beans (13). Lugger (20), in 1896, reported a serious outbreak of the insect on beans in Minnesota. During the summer of 1924, members of the Division of Economic Entomology of the University of Minnesota observed several severe infestations of beans. It is said to be distributed generally throughout the United States, Canada, and Europe (5). It has been reported from decaying potato plants or "seed potatoes" from New York (28), Louisiana (30), Florida (29), Idaho, Maryland, and Virginia (4).

Sellards (29) reports the insect very destructive to potatoes in Florida in 1905, and describes its attack as follows:

"The decay of the potatoes was found to be more severe on the low wet lands. The maggots were present on the higher lands, although in smaller numbers. The attack on the potatoes is made from the cut surface. The maggots seem to prefer decaying potatoes, but attack perfectly sound ones. Starting from the cut surface, tunnels are run in various directions through all parts of the potato. In some cases the potatoes were found much decayed and a mass of maggots, hardly anything of the potato remaining but the outer skin."

Hylemyia trichodactyla Rond, a species almost indistinguishable from the seed-corn maggot and which is known as the seed-potato maggot, has been reported from potato plants in Aroostook County, Maine (17) and from turnips in Ontario (12). The writer has not recognized this species in Minnesota.

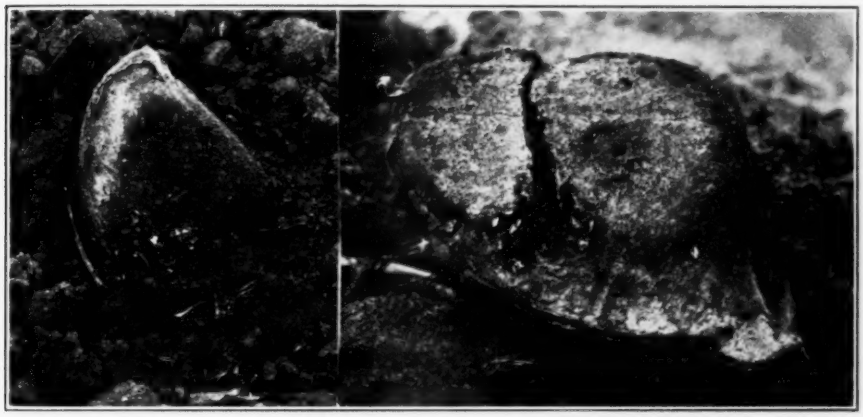

FIG. 4. Eggs of the seed-corn maggot on, and in the soil near, potato seed pieces. Photographed three days after planting. These seed pieces had been planted by machine in the usual way, but were incompletely covered. The eggs were mostly on the under side of the seed pieces, which were turned over before the eggs were discovered.

LIFE HISTORY

Eggs.—Like the cabbage-maggot fly, the adult female of the seed-corn maggot deposits eggs on, or in, the soil near freshly planted seeds or young sprouting seedlings. The early observations of the writer (19), which were made several weeks after planting time in 1924, led him to think that the eggs were deposited on potato seed pieces before planting, but further observations in 1925 showed quite clearly that the eggs are deposited most frequently on, or in, the soil near seed pieces after planting (Fig. 4), and

even after the sprouts have appeared above the ground. Evidence of egg deposition in the soil before the seeds of beans were planted has been reported from New Jersey (13). The eggs are small, slightly less than 1 mm. in length, but may be seen readily with the naked eye. They are slightly curved, white, and distinctly reticulate (Plate XV, b and c). When freshly deposited, they are covered with a sticky fluid, which causes them

FIG. 5. Soil cracks along the top of a potato row three days after planting. Eggs of the seed-corn maggot were found several inches below the surface of the soil in these cracks. The photographs in fig. 4 and this one were taken in the same field on the same day. There was more than 10 per cent blackleg in the field when observed about two months later.

to adhere to the soil particles or anything with which they may come in contact. The writer has frequently observed the adult female deposit eggs in the soil near exposed seed pieces. With her hind legs the female pushes aside small clods of earth until her ovipositor is several mm. beneath the

surface, and the eggs then are deposited, usually in direct contact with moist soil.

In 1925 the adult flies were very abundant as early as May 2 in certain potato fields in northern Minnesota. Potato planting did not begin in this section until about May 10. The first eggs observed were found on May 13, and the height of the egg-laying period was reached about the 25th and 26th of May. During this period many eggs were obtained for experimental purposes by placing freshly cut seed pieces on the surface of the soil in potato fields and leaving them exposed for twenty-four hours. On May 21 more than 500 eggs were obtained from the soil in immediate contact with less than 100 seed pieces which had been exposed not more than twenty-six hours. In many instances, egg deposition has been observed in less than ten minutes after exposing the seed pieces.

FIG. 6. Three puparia of the seed-corn maggot in the soil, adhering to a decayed seed piece from a blackleg-infected plant. These puparia were close to the seed piece, although they are frequently found several inches away.

The soil in the Red River Valley contains a great deal of colloidal material and is very subject to cracking. Almost any freshly turned soil cracks abundantly upon drying, although the soil may remain flocculent and friable. It is not uncommon for rather large cracks to develop along the ridge of soil left by the potato planter. These cracks are frequently several inches deep, and are directly over the planted seed pieces (Fig. 5). Flies were observed near these cracks, and, on careful examination, eggs were found as deep as four inches beneath the surface of the soil. Eggs also have been found several inches beneath the surface of the soil in cracks made by the sprouting seed piece as it breaks through the ground.

Larvae.—The eggs hatch in about three days after they are deposited. The maggot, when it emerges, is nearly transparent and only slightly longer than the egg, but it is able to move about rapidly in search of food. The action of freshly-hatched maggots was observed by placing one-half of a

sound, freshly-cut tuber and several eggs in contact with the wall of a glass jar and filling the jar with moist soil. Most of the eggs hatched within twenty-four hours. The young maggots had no difficulty in finding the seed piece, although some of the eggs were more than an inch from the tuber. For the first twenty-four hours after hatching, the maggots crawled over the cut surface of the tuber. At the end of this period a profuse growth of bacteria was evident. During the next two days most of the maggots disappeared into the interior of the tuber, which was completely decayed in less than a week. The remaining half of the same tuber, kept under similar conditions but not attacked by maggots, remained healthy.

The mouth of the maggot is equipped with two black, sharp, claw-shaped structures (Plate XV, j), with which it is able to tear apart the tissues of the

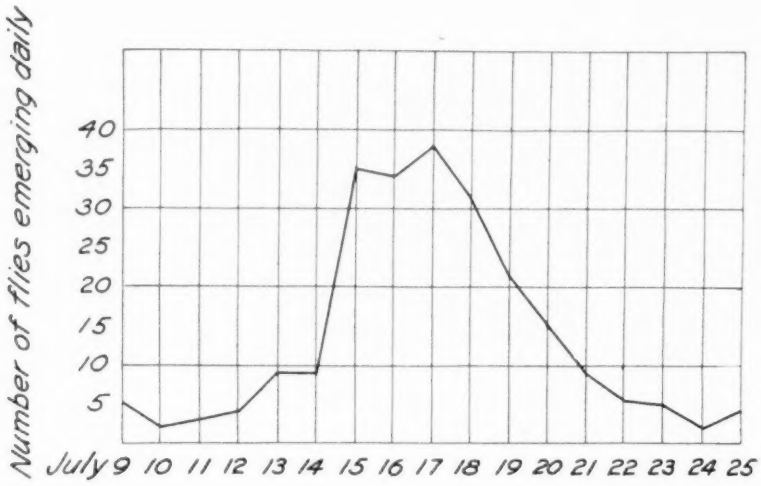

FIG. 7. A graph showing the time of emergence of 231 adult seed-corn-maggot flies from puparia collected from blackleg-infected plants in 1924.

seed piece, and which no doubt serve as very effective means of inoculating the seed piece with bacteria. It is obvious that, with maggots present, any cork laid down by the tuber would be quickly destroyed and would not be effective in checking the decay.

Puparia.—The maggots enlarge very rapidly, and in from fifteen to twenty-five days reach a maximum length of about 7 or 8 mm. (Plate XV, f). When this size is reached, they leave the decayed seed piece, enter the soil, and pupate (Fig. 6). Puparia frequently are found four or five inches from the seed piece, although most of them remain within a radius of three inches or less. In nature, pupation begins about one month after the pota-

toes are planted, and frequently it is under way when the first symptoms of blackleg are evident. In a field planted on the 28th of May, 1924, the first blackleg-infected plants were observed on June 29, when the normal plants were from six to eight inches high. Puparia also were found at this time; and, by July 4, puparia were more abundant than larvae. Many plants which manifested the first symptoms of blackleg after this date were examined, and almost invariably it was found that pupation already had occurred. This early pupation may account for the fact that the insect has been so commonly overlooked.

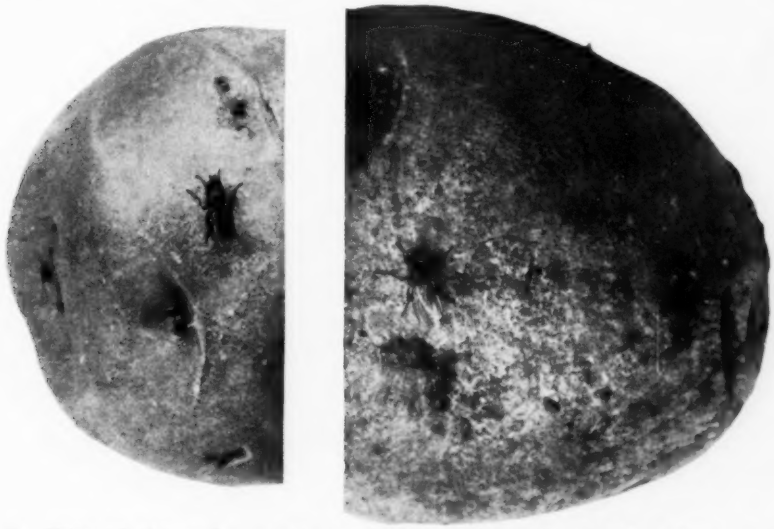

FIG. 8. Flies of the seed-corn maggot (female, left; male, right) resting on potato tubers—a very common sight in Minnesota potato fields at planting time.

The puparia (Plate XV, h) are from 4 to 5 mm. in length, and vary in color from light brown to dark reddish-brown. The duration of this stage varies from seven to fourteen days. Fig. 7 shows graphically the period of emergence of 231 flies from puparia collected in 1924.

Adult flies.—The adults, which for some unknown reason emerge from the puparia mostly at night, are able to work their way through several inches of soil. When freshly emerged, they are lighter in color than normal, and their wings are not unfolded. Within a few hours, however, they take on their normal gray color and their wings unfold. Adult flies are shown in figure 8, and Plate XV, a and d. A detailed description of the adults will not be given here, as it can be found readily in entomological literature. Mention should be made, however, of the usual means of distinguishing them from other closely related species. On the tibia (middle

segment) of each hind leg of the male, there is a row of from 21 to 24 short, slightly curved bristles (Plate XV, g). These bristles distinguish the seed-corn maggot from all other species of the same genus, except the seed-potato maggot. It is often very difficult to distinguish the females from those of closely related species.

FIG. 9. Secondary infection of blackleg disseminated and inoculated by the seed-corn maggot. A young maggot was found working at the base of the cluster of roots indicated by the arrow. The infection was entirely confined to the cortex. The pith was not affected. The dark spot at the base of the stem is a lesion caused by *Rhizoctonia solani*. The negative was much under-exposed.

Broods.—The number of broods of this insect are not definitely known (8). At least two broods have been observed by the writer in Minnesota. The second brood eggs are deposited most frequently near the ground line of potato plants affected with blackleg, and the maggots develop in the stems of such plants. In only a few cases have the maggots of the first brood been observed to pass upward into the stems. A considerable number of the second brood eggs are deposited also at the base of the healthy potato plants, which later become diseased. Several well marked cases of blackleg have been observed which were without doubt due to this type of secondary infection (Fig. 9).

The insects are supposed to hibernate as puparia in the soil. Evidence of this was observed in the early spring of 1924, when adult flies were very abundant in fields which were known to have been heavily infested the previous year, and none could be found in neighboring fields known to

have been free from blackleg. Observations indicate that the insect usually does not hibernate in potato cellars or piles of refuse potatoes. Many species of scavenger flies have been observed breeding in piles of refuse potatoes, but the seed-corn maggot never has been found developing in such places.

FIG. 10. Young potato plants decayed as a result of inoculating the stems with a broth culture of bacteria obtained from the tracks made by freshly hatched maggots on sterile agar.

THE RELATION OF THE INSECT TO THE BLACKLEG DISEASE

Pathogenic bacteria commonly present on eggs

Although the close association of the seed-corn maggot with potato blackleg is highly suggestive, it does not in itself prove that the insect is an agent of dissemination of the disease. In order to determine whether the insect actually spreads the pathogene, a number of eggs were collected and placed upon sterile agar, or on sterile glass near sterile agar. Upon hatching, the maggots crawled rapidly about over the agar, leaving minute tracks where their mouth parts had penetrated the agar. Within a few hours of the passage of the maggot, an examination showed colonies of bacteria developing along the tracks (Plate XV, i). In general, each colony coincided with a puncture of the agar by the mouth parts of the maggot. Broth cultures made from these colonies, when inoculated into tubers and

on young potato plants, were pathogenic (Fig. 10).[4] After this experiment had been repeated several times, with similar results in the majority of cases, it was evident that the eggs were commonly contaminated with pathogenic bacteria.

FIG. 11. Potato tubers and a carrot slice six days after inoculating with a broth culture of bacteria obtained by aseptic dissection of the abdominal contents of adult flies caught on May 5, 1925.

PATHOGENIC BACTERIA PRESENT IN BODY OF ADULT FLIES

Since the eggs commonly bear pathogenic bacteria, it seemed probable that the bacteria would be present also in the body of the insect. Therefore a number of flies were caught, chloroformed, and dissected aseptically. The contents of the abdomen were carefully removed with sterile needles and placed in a tube of broth or on an agar slant. Cultures obtained in this way also proved to be pathogenic (Fig. 11).

Pathogenic bacteria were obtained also from the excrement of flies deposited on the inside of a sterile culture tube. These preliminary experiments were performed during July, 1924. They were repeated on a larger scale during the first week in May, 1925, using flies of the first brood to emerge in the spring, and obtained before potato planting had been started. The results are given in tables 1 and 2.

PASSAGE OF BACTERIA THROUGH PUPAL STAGE

The constant association of pathogenic bacteria with the various stages of the insect suggested the possibility of biologic transmission, with the

[4] Further study of these cultures showed that they were nearly always mixed cultures of at least three species of bacteria. During the course of the work, a considerable number of apparently different species, or perhaps strains, of bacteria have been isolated from the insect or from plants attacked by the insect. No attempt has yet been made definitely to identify them, it being considered sufficient, for the present, to determine whether they were pathogenic.

TABLE 1.—*Results of aseptically dissecting adult seed-corn-maggot flies, and culturing the abdominal contents*

Fly No.	Sex	Culture No.	Growth[a]	Pathogenicity to potato tubers[b]
1	Female	A	–	
		B	+	–
2	Female	A	+	Not tested
		B	+	Not tested
5	Female	A	+	+
		B	+	Not tested
6	Female	A	+	+
8	Female	A	+	+
9	Female	A	+	+
		B	+	+
10	Female	A	+	+
11	Female	A	+	+
13	Female	A	+	+
3	Male	A	+	+
7	Male	A	–	
12	Male	A	+	–
15	Male	A	+	Not tested
16	Male	A	–	
17	Male	A	+	+

[a] –, no growth
+, growth
[b] –, not pathogenic
+, pathogenic

TABLE 2.—*Pathogenicity of bacterial cultures from excrement of flies deposited on sides of sterile culture tubes*

Fly No.	Sex	Growth	Pathogenicity
1	Female	+	Not tested
2	Female	+	+
4	Female	+	+
6	Female	+	+
7	Female	+	–
3	Male	–	
5	Male	+	+

bacteria a constant symbiont with the insect. A number of such symbiotic relationships between insects and microorganisms have been reported in recent years.

Guyénot (10), in 1907, studied the relationship between *Micrococcus flavus liquefaciens* (Flugge) and *Phormia regina* Meigen (a scavenger),

and concluded that there was a true symbiotic relationship between the two organisms. The bacteria, which were always found with the larvae, were shown to be the agents of liquefaction of the food material of the larvae, while the larvae aided the development of the bacteria by the action of their hook-like mouth parts.

Baumberger (2) has shown a similar relationship between *Drosophila* and yeast, but in this case the yeast serves as a food for the larvae and not merely as an agent of liquefaction. He thinks, too, that the larvae studied by Guyénot may also feed upon the bacteria. He concludes that "The experiments and considerations all tend to establish the principle that insects inhabiting fermenting and decaying substrata of low protein content, usually feed upon the microorganisms present and thus benefit by the power of the fungi to extract, absorb, and synthesize many non-protein nitrogenous compounds." Delacourt and Guyénot (6) and Guyénot (11) also obtained results with *Drosophila* similar to those obtained by Baumberger.

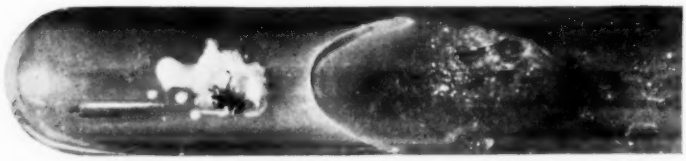

FIG. 12. Bacteria developing from an adult fly 48 hours after emerging from a surface sterile puparium. The fly became wedged between the agar and the side of the tube soon after emerging. Bacteria developed only where the fly had touched the agar. The agar immediately around the pupal skin remained sterile.

Baumberger was able to obtain sterile adults of *Drosophila* by surface sterilization of the puparia with fifty per cent alcohol, indicating that the yeast did not persist inside of the puparia. He was unable, however, to sterilize the pupae of *Musca domestica*.

Bacot (1), working with *Musca domestica*, concludes:

"1. Pupae and imagines of *Musca domestica* bred from larvae infected with *B. pyocyaneus* under conditions which exclude the chance of re-infection in the pupal or imaginal period undoubtedly remain infected with the bacillus.

"2. In the imago the infection is maximal at emergence and then diminishes suddenly.

"3. The possibility of a dangerous pathogenic microorganism being taken up by the larva and subsequently distributed by the fly is one which deserves serious consideration."

The same author, in a footnote, announces the isolation of *B. typhosus* from the interior of pupae, the larvae of which had been fed on the organ-

ism. Graham-Smith (9) obtained cultures of *B. anthracis* from 26 of 51 flies reared from larvae which had fed on meat infected with the organism.

Buchner (3), and Hertig and Wolbach (15), have described several cases of symbiosis between insects and microorganisms, in some of which the microorganisms always were found associated with the insect in all of its stages, including the egg.

In order to determine whether the bacteria associated with the seed-corn maggot could remain alive within the puparium, twelve puparia were surface sterilized in July, 1924, in the following manner: they were first soaked in 5 per cent alcohol for 5 minutes; then in an aqueous solution of mercuric bichloride (1–1000) for 3 minutes; then rinsed in 50 per cent alcohol for about 1 minute, and placed on a plate of soft beef extract agar. After 48 hours bacterial growth had developed around two of the puparia. After 72 hours the remaining ten puparia were removed, and each was placed in a separate tube of sterile beef-extract broth. After 24 hours the broth in every tube was still clear, and remained so until discarded several days later. The puparia were then transferred to new tubes of sterile broth. these also remained clear for 24 hours. The puparia were then crushed with sterile forceps. In every tube the broth became cloudy with bacterial growth within 12 hours. Transfer cultures from two of these tubes were tested and, although evidently mixed, proved pathogenic to tubers.

This experiment shows that the bacteria may remain alive within the puparia, but does not prove that they remain alive until the adult emerges. Therefore, in July, 1925, when another supply of puparia was available, this point was tested. Forty puparia were surface disinfected in the following manner. They were soaked in 50 per cent alcohol for 3 to 5 minutes, in a mercuric bichloride solution (1–1000) for 3 to 5 minutes, and again in 50 per cent alcohol for 3 to 5 minutes. Each puparium was then rinsed in a tube of sterile broth for 5 to 10 minutes. They were then removed from the broth with a sterile needle and, without rinsing, placed

TABLE 3.—*Results of experiments to determine the presence of pathogenic bacteria in adult flies emerging from surface sterilized puparia*

No. of puparia disinfected	No. proved surface sterile	No. adults emerging	No. tubes showing bacterial growth 24 hours after emergence of fly	No. cultures pathogenic
40	28	25[a]	25	9 out of 12 tested

[a] The three remaining puparia were crushed on sterile agar, and abundant bacterial growth developed from each puparium in twenty-four hours.

on agar slants in culture tubes, where they were allowed to remain until the adult flies emerged. The results are given in table 3, and illustrated in figure 12.

This experiment seems to show conclusively that the bacteria remain alive within the puparium and emerge with the adult fly in an active and virulent state.

BACTERIA NOT INSIDE OF THE EGGS

In several cases [Buchner (3), and Hertig and Wolbach (15)], microorganisms living symbiotically with insects have been shown to live inside of the egg. It seemed desirable to determine if this were true in this case. It was decided, therefore, to attempt to surface sterilize a number of eggs. Much to the surprise of the writer, it was found that this could be done very

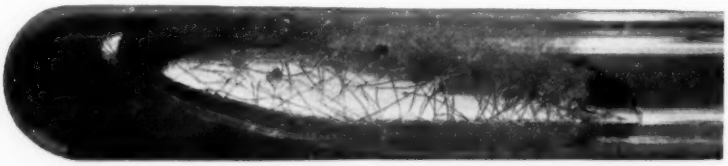

FIG. 13. A culture tube of sterile agar showing tracks made by a sterile maggot hatched from a surface sterilized egg. Photographed by reflected light one week after maggot emerged from the egg.

easily without injury to the viability of the egg. The uninjured shell of the eggs is very impermeable to an aqueous solution of mercuric bichloride. It was found that the eggs could be soaked in such a solution (1–1000) for as long as two hours without injury to the embryo. Almost one hundred per cent surface sterility was obtained in this way. The surface sterilized eggs, after being rinsed in sterile broth, were placed on agar slants. The maggots which hatched from such surface sterilized eggs invariably were sterile, showing conclusively that the bacteria are not carried within the eggs. The results of a number of these treatments are given in table 4. Figure 13 shows an agar slant marked with tracks of a sterile maggot photographed one week after the maggot had emerged from the egg. The agar was absolutely sterile.

The above results were confirmed by aseptic dissection of the eggs from the bodies of female flies. The eggs obtained in this way were placed separately on the surface of sterile agar. Those remaining free from bacterial growth after seven days were removed and crushed in a tube of sterile broth or on an agar slant. In every case the broth remained sterile.

PROBABLE METHOD OF SURFACE CONTAMINATION OF EGGS

The results presented in table 4 show that the bacteria are not carried within the egg. The question of how the eggs become contaminated nat-

TABLE 4.—*Results of experiments to determine the presence of bacteria inside of eggs of the seed-corn maggot*

Egg no.	Treatment	Time	Surface sterility	Viability	Maggot sterility
1	Mercuric bichloride 1–1000	1 min.	Sterile	Viable	Sterile
2		2 min.	do	do	do
3		3 min.	Not sterile	do	Not sterile
4		15 min.	Sterile	do	Sterile
5		do	do	do	do
6		do	do	do	do
7		do	do	do	do
8		do	do	do	do
9		do	do	do	do
10		do	do	do	do
11		do	do	do	do
12		do	do	do	do
13		do	Not sterile	do	Not sterile
14		1 hr. and 45 min.	Sterile	do	Sterile
15		do	do	do	do
16		do	do	do	do
17		do	do	do	do
18		do	do	do	do
19		do	do	Not viable	
20	Hot formaldehyde 1 pt. to 15 gallons	2 min.	Not sterile	Viable	Not sterile
21		do	do	do	do
22		do	do	Not viable	
23		do	Sterile?	Viable	Not sterile
24		do	do?	do	do
25		do	Sterile	Not viable	
26		do	do	do	
27		do	do	do	
28		do	do	do	
29		do	do	do	
30		do	do	do	
31[a]		do	do	do	

[a] Many more were later surface sterilized in $HgCl_2$ 1–15, a record of which was not kept, but all surface sterile eggs which hatched gave rise to sterile maggots.

urally arises. A study of the anatomy of the adult female fly shows how this very probably takes place. The end of the ovipositor and anal tract are shown in Plate XV, e. The openings through which the excrement and the eggs pass are in close proximity, and are surrounded by bristly segments. The eggs are moist and covered with a gelatinous substance when deposited, and, since the excrement of the fly contains the bacteria, it would appear very difficult for the eggs to be deposited without becoming contaminated. Maggots in the act of hatching have been observed, and they always crawl

over the old shell for a few moments after emerging. It is very probable that the maggot gets the bacteria into its system at this time.

BACTERIA NECESSARY FOR DEVELOPMENT OF LARVAE

Baumberger (2) has shown that *Drosophila* living in fermenting fruit are dependent on yeast cells for their food supply and that sterile larvae are unable to grow normally on sterile fruit. They grow well, however, with the addition of living yeast. In the experiment reported above, in which sterile larvae of the seed-corn maggot were obtained by surface sterilizing the eggs, it was observed that the few which became contaminated with bacteria grew and increased in size rapidly, although they did not pupate normally; while those remaining sterile did not increase appreciably in size, although they remained alive for more than a week. Bacteria were added to a few of these after they had remained about five days on sterile agar. Within forty-eight hours they had more than doubled in size. Since these maggots were growing on slants of beef-extract agar, it was decided to repeat the experiment, using sterile and non-sterile potato plugs as media. A quantity of eggs was obtained, surface sterilized, and placed on sterile agar. After the maggots emerged, they were allowed to remain on the sterile agar for twenty-four hours, to determine whether they were sterile. Forty-eight sterile maggots were obtained in this way. As soon as proof of sterility was obtained, they were removed to sterile media, as indicated in table 5. One half of the tubes were inoculated with pathogenic bacteria; the rest were allowed to remain sterile. The results are given in table 5, and illustrated in Plate XV, k. They show clearly that the presence of bacteria is necessary for the normal growth of the larvae when feeding on potato tubers. They indicate, however, that the potato furnishes something essential to normal growth, since the larvae growing on inoculated beef extract agar did not pupate and emerge normally.

THE PRODUCTION OF THE DISEASE BY "INOCULATING" SEED PIECES WITH EGGS OF THE SEED-CORN MAGGOT

In the spring of 1925, attempts were made to produce the disease by placing eggs in the soil near the seed pieces. Eggs were collected from the soil near seed pieces which were exposed on the surface of the ground. A total of 900 seed pieces were "inoculated." Holes about four inches deep were dug in the soil, and a freshly cut seed piece was put in each hole. From 3 to 5 eggs were placed in the soil near each seed piece and covered with about four inches of loose soil. Nine rows of 100 "inoculated" seed pieces and 50 "uninoculated" seed pieces were planted. Notes were taken six weeks after planting. Of the 900 "inoculated" plants, 46, or a little

TABLE 5.—*Results of experiments to determine the relation of bacteria to the development of the larvae of the seed-corn maggot*

Sterile maggot No.	Medium	Result	Sterile maggot No.	Medium	Result
1	Sterile potato plug	Very little growth, maggot remaining transparent and dying after 10 to 20 days	1a	Inoculated potato plug	Normal growth; pupated and emerged
2	do		2a	do	do do
3	do		3a	do	do do
4	do		4a	do	do do
5	do		5a	do	do do
6	do		6a	do	Pupated early; did not emerge[a]
7	do		7a	do	Dead before pupating; good growth
8	Sterile beef extract agar		8a	Inoculated beef extract agar	Grew slowly; pupated early
9	do		9a	do	do do
10	do		10a	do	do died before pupation
11	do		11a	do	do do
12	do		12a	do	do do
13	do		13a	do	do do
14	do		14a	Inoculated potato plug	Normal growth; pupated and emerged
15	Sterile potato plug		15a	do	do do
16	do		16a	do	do do
17	do		17a	do	do do
18	do		18a	do	do do
19	do		19a	do	do do
20	do		20a	do	do do
21	do		21a	do	do do
22	do		22a	do	do do
23	do		23a	do	do do
24	do	Normal growth; pupated and emerged[b]	24a	do	do do

[a] Some of the potato plugs with maggots and bacteria were to moist for good growth, probably explaining the action of those which did not pupate and emerge normally.

[b] Accidental contamination.

more than 5 per cent, developed typical blackleg, while there was not a single blackleg plant in the checks. There were also 45 skips in the "inoculated" rows against 4 skips in the checks. The results, although not very striking, show that blackleg may be produced by "inoculating" the seed pieces with eggs of the seed-corn maggot.

The writer is convinced that, with a different method of "inoculation" and more favorable weather conditions, a much higher percentage of diseased plants can be obtained. At the time this experiment was started, it was believed that in nature many of the eggs were deposited on the seed pieces before planting. If this were true, the method would have been somewhat comparable. However, later observations showed that in all probability most of the eggs are deposited after planting and much nearer the surface of the soil, or at least in open cracks. In the methods used, the eggs were under about four inches of soil which was packed tightly by heavy rains[5] shortly after planting.

POSSIBILITIES OF CONTROL

The problem of control is a difficult one, and no definite recommendations can be given at this time. Since it has been found that most of the eggs are deposited in the soil after planting, it is evident that protection of the seed pieces before planting is not sufficient. That the eggs are very resistant to mercuric bichloride and hot formaldehyde is shown by the data given in table 4. However, the larvae of closely related insects are known to be very susceptible to mercuric bichloride. Some preliminary experiments indicate that seed treated after cutting with a solution of mercuric bichloride 1–1000 and allowed to dry retain sufficient mercury to kill maggots which attack it. Seed pieces treated with hot formaldehyde were readily attacked by the maggots and decayed rapidly. That the latter treatment was in no way injurious to the maggots was indicated by the fact that they reached normal size, pupated, and emerged as adult flies. This may explain the fact that mercuric bichloride treatments in many cases have given better control of blackleg than formaldehyde treatments. More experiments are necessary, however, before any definite conclusions can be reached.

DISCUSSION

The observations and experiments reported above show conclusively that plant pathogenic bacteria are constantly associated with the seed-corn mag-

[5] The rainfall at this station was extremely heavy during the five weeks immediately following the planting of the potatoes in this experiment. The official government Weather Bureau station located at the Northwest School and Station, Crookston, reported rain on 27 days between May 18, the first date of planting, and June 30. A rainfall of 3.96 inches fell on May 23.

got in the various stages of its development, and that potato blackleg[6] may develop following the attack of the larvae on otherwise healthy seed pieces. From the standpoint of the development of blackleg, the insect acts not only as an agent of dissemination but as an agent of inoculation. It has been shown that, although seed pieces may be partly decayed by the blackleg pathogene, they may be able to cork off the decay and produce a healthy plant if planted under reasonably good growing conditions. On the other hand, if a seed piece is attacked by a seed-corn maggot, it is unable to check the decay. Wound cork formation is not sufficient to overcome the combined action of the maggot and pathogenic bacteria. Any cork that is formed is soon destroyed by the maggot, which at the same time hastens the development of decay by spreading the bacteria throughout the tissues of the tuber.

It should be pointed out here that the experiments reported in this paper dealing with artificial inoculation have no bearing on natural infection of tubers through the stolons of diseased plants. This means of infection constitutes an entirely different phenomenon, which will be discussed in a subsequent paper.

It also should be understood that dissemination by the seed-corn maggot is not considered to be the only means of dissemination of blackleg. Moreover, the writer is of the opinion that in many cases maggots may be found in the seed pieces of plants which would have produced blackleg in the absence of the insect. In fact, the possibility that the insect attacks only decaying plants has been suggested (28), and was seriously considered, but the observations on the egg-laying habits and the decaying action of young maggots when placed on perfectly sound seed pieces, together with the constant association of pathogenic bacteria with the insect, convinced the writer that the maggots could, and commonly do, attack perfectly healthy seed pieces and inoculate the plants with blackleg. In Minnesota the presence of this insect will explain many, but probably not all, of the outbreaks of blackleg in fields planted with seed known to be free from blackleg infection.

The possibility of infection from this source should always be taken into consideration in field experiments on blackleg. It is very likely that many cases of blackleg infections which, in past experiments, have been interpreted as seed piece dissemination may have been due to attacks of the seed-corn maggot. It also must be considered in judging the results of seed potato certification.

In view of the changes that are known to take place in the puparium prior to the emergence of the adult, it was very surprising to find that the

[6] The term "blackleg," as used here, refers to a bacterial necrosis of the stems of potato plants answering the general descriptions of that disease as described in the literature, without implying that the condition is caused by any particular species of bacteria.

bacteria passed through this stage and emerged with the adult fly unharmed. It would be interesting to follow the bacteria through this stage by histological methods. Although material has been fixed and imbedded, this has not yet been done.

The discovery of the relation of this insect to potato blackleg constitutes, insofar as the author knows, a distinctly new type of insect transmission of a bacterial plant disease. It is biological transmission in the sense used by Rand and Pierce (26), although in animal pathology the term "biological" generally is used only when the insect constitutes the only known means of transmission.

The facts are very suggestive as to the relation of other similar insects to bacterial and possibly fungous diseases of plants. It may be of interest to mention that most of the data presented here for the seed-corn maggot and potato blackleg have been found to hold true for the cabbage maggot and the soft rot which usually accompanies its attack.

The relationship between the insect and the bacteria should, in all probability, be classed as symbiotic. It has been shown that the bacteria are not only beneficial to, but necessary for, the development of the larvae. Likewise, it is obvious that the insect contributes to the welfare of the bacteria by disseminating them, by inoculating them into the plant, by aiding them in destroying the plant tissue, and by providing them with a sure means of hibernation.

The relationship is of interest in considering the origin of the parasitic habits of this type of insect. Frost (7), in his monograph on the leaf mining diptera of North America, states: "It is possible that the scavenger habit developed before the plant-feeding habit, the larvae having first gained entrance to the plant through decayed roots and stems. Some writers would take exception to this hypothesis, contending that insects first became established on living material and, as the host died, became adapted to a saprophytic, or scavenger habit. It seems plausible, however, that in the leaf-miners the adaption is working in the reverse direction, and that the scavenger habit is the more primitive. According to this hypothesis, the larvae, having first entered the plant as scavengers, became adapted to an existence within the living host and finally migrated to the stems and leaves where they are now known as leaf-miners." Following this same line of thought, it would seem probable that, during the evolution of the insects, certain scavengers developed the habit of depositing their eggs in plants attacked by certain plant pathogenic bacteria until these organisms were included among their internal symbionts. With plant pathogenic bacteria as symbionts, maggots from eggs deposited on healthy plants would be able to induce the decay and thus survive. In this way the parasitic habit may

have arisen. On the other hand, when we consider that the association with the bacteria enables the insect to shorten its life cycle (2), it may be argued that this is the most highly developed state. The writer must confess that he does not have enough information about the habits of other species of insects on which to base an opinion. Attention is called to the possibilities because of their evident bearing on a very interesting problem. More extensive investigation of the food habits of such insects is desirable.

SUMMARY

1. Investigations of numerous outbreaks of potato blackleg in Minnesota showed that much of the disease could not be accounted for by seed piece transmission.

2. Attempts to produce the disease by inoculating the seed pieces were unsuccessful because of the striking ability of the sprouting seed piece to cork off the decay. These results indicate that contamination of the seed piece at the time of cutting usually is not an important factor in the dissemination of the pathogene.

3. Observations in Minnesota extending over three years revealed a close association between the seed-corn maggot (*Phorbia fusciceps* Zett.) and potato blackleg.

4. The seed-corn maggot is known to be parasitic on a wide range of host plants, including potatoes; and a soft rot of attacked tissues characteristically follows its injury.

5. Eggs of the insect are deposited on, or in, the soil near healthy seed pieces or sprouts.

6. The eggs are commonly surface contaminated with plant pathogenic bacteria.

7. The larvae inoculate the seed pieces with the bacteria and aid the development of the disease by destroying or preventing the formation of wound cork.

8. The bacteria are constantly associated with the insect, passing through the pupal stage, and emerging with the adult fly in a virulent state. Pathogenic bacteria are commonly present in the intestinal tract of adult flies.

9. The bacteria do not occur inside of the eggs. The surface of the eggs probably becomes contaminated at time of deposition, when, coated with a sticky fluid, they pass between the anal appendages.

10. Sterile maggots, obtained from surface sterilized eggs, were not able to grow on sterile potato tubers but grew normally when bacteria were added, showing that the bacteria are essential for the development of the maggots.

11. The maggots developed slightly better on inoculated potato plugs than on inoculated beef-extract agar.

12. Typical blackleg was produced by "inoculating" healthy seed pieces with eggs of the seed-corn maggot.

13. The seed-corn maggot is an agent of dissemination, inoculation, and hibernation of plant-pathogenic bacteria.

14. The association between the bacteria and the insect appears to be one of mutualistic symbiosis, and constitutes a type of insect transmission of a bacterial plant disease not previously described.

15. The facts presented here may have a bearing on the problem of the origin of parasitism among dipterous insects.

16. No measures for the control of the insect are recommended, although preliminary experiments indicate that a thin coating of mercuric bichloride over the surface of cut seed pieces may be effective in preventing injury.

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EXPLANATION OF PLATE XV

a. Adult female seed-corn-maggot fly. (Approx. 10 ×.)

b. Eggs of the seed-corn maggot. (Approx. 50 ×.)

c. An egg of the seed-corn maggot. (Approx. 100 ×.)

d. Adult female seed-corn-maggot fly. (Approx. 1¾ ×.)

e. Tip of ovipositor of adult fly showing the bristly segments surrounding the anal opening and the opening through which the eggs pass. Surface contamination of the eggs probably takes place here.

f. A larva of the seed-corn maggot almost fully developed. (Approx. 1½ ×.)

g. A hind leg of a male fly of the seed-corn maggot, showing the row of bristles along the middle segment which is a characteristic of this species.

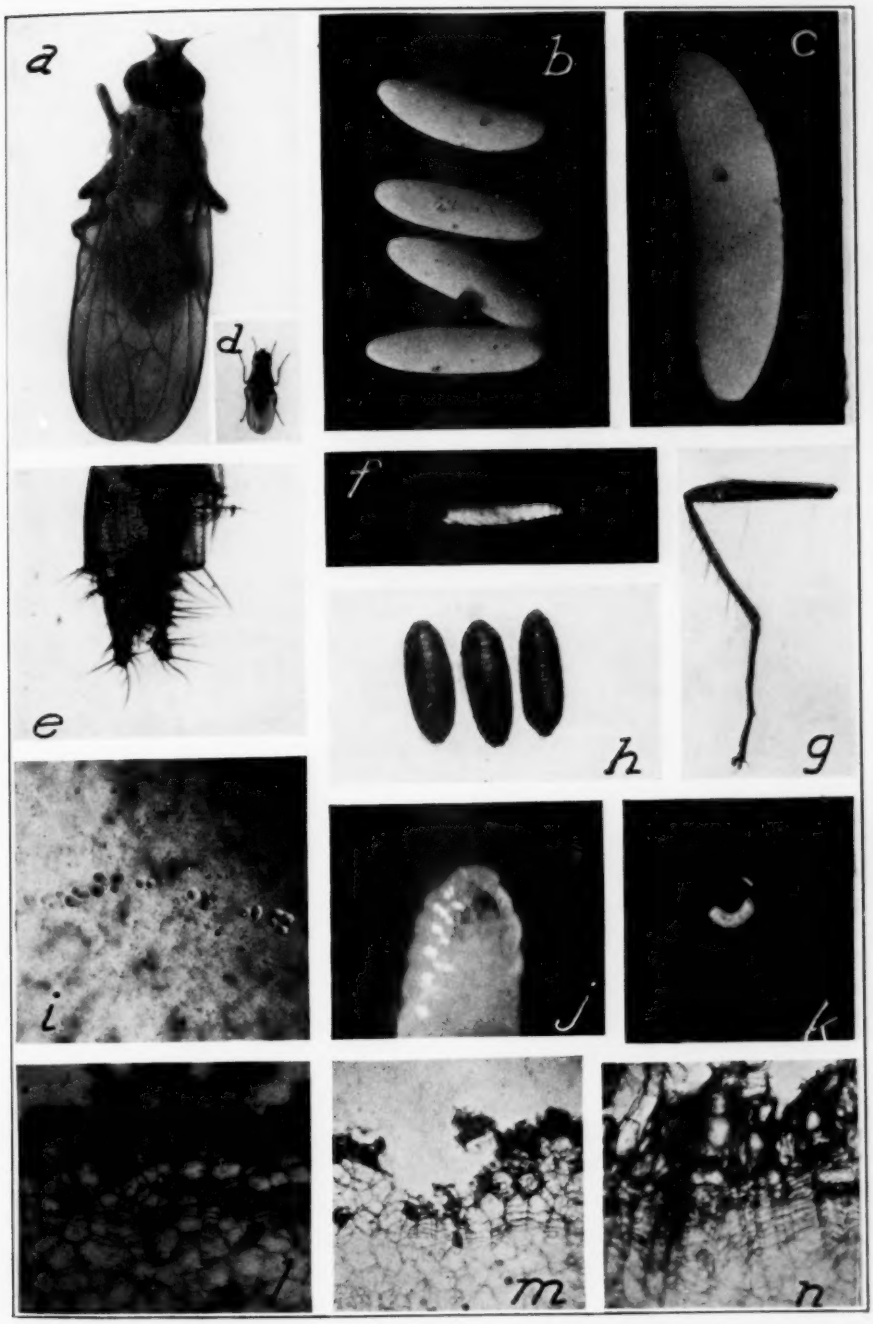
h. Three puparia of the seed-corn maggot. (Approx. 3 ×.)

i. Bacterial colonies developing in the tracks of a maggot freshly hatched on sterile agar.

j. The mouth parts of a seed-corn-maggot larva, showing the sharp claw-like structures with which it attacks and burrows into plant tissue.

k. Two larvae hatched from surface sterile eggs. The upper and small one had fed for twelve days on a sterile potato plug. The larger one fed for twelve days on a potato plug inoculated with plant pathogenic bacteria.

l, m, and n. Sections through the healed surface of inoculated seed pieces about two months after planting, showing the cork layer which has completely and effectively walled off the bacteria, thus preventing further decay.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PSEUDOMONAS (PHYTOMONAS) PISI SACKETT, THE CAUSE OF A POD SPOT OF GARDEN PEAS

C. A. LUDWIG

INTRODUCTION

Pseudomonas pisi Sackett (*Phytomonas pisi* (Sackett) S. A. B.),[1] which was described as the cause of a stem blight of field and garden peas in Colorado, has recently been found to produce a pod spot also.

When the spots are young they have the usual translucent, water-soaked appearance characteristic of bacterial lesions; but they usually become yellowish, and finally brown, as the pods mature. When the pods are ripe, both the healthy and diseased tissues have the characteristic stiff papery texture of pea pods. The brown color of the spots, however, sets them off, in strong contrast, from the lighter color of the healthy tissue (Plate XVI, 5). If young pods are infected severely, they shrivel up and die. The diseased tissues swarm with bacteria. The symptoms on the other parts of the plant have been described by Sackett[1] and in the Plant Disease Reporter,[2] and will not be listed here.

ISOLATION OF THE ORGANISM

The organism used in these studies was isolated by the usual poured plate method[3] from a specimen of spotted pods received in April, 1922, from Mr. Rudolph Farmer, then horticultural specialist on the Extension Division staff of Clemson College. They came from a planting near Latta, South Carolina; and there was no report of any stem blight accompanying or preceding the spots. The chief commercial damage was in the appearance of the pods, which affected the marketability of the crop.

INOCULATIONS

The results of the inoculations are given in table 1. Checks, similarly treated except for inoculation, remained healthy in all instances. Except as noted, the inoculations were made in the greenhouse.

[1] Sackett, W. G. A bacterial stem blight of field and garden peas. Col. Agr. Exp. Sta. Bul. 218. 1916.

[2] U. S. Dept. Agr. Plant Disease Reporter 8: 13–14. 1924.

[3] These plates were poured by Mr. W. B. Aull, associate professor of bacteriology. Assistance in this work from the following is acknowledged also: Mrs. W. B. Aull, Mr. F. O. Myers, and Mr. J. H. Hunter.

Inspection of table 1 will show that most of the inoculation work for the first three years has been grouped by seasons and recorded in general terms instead of taking each inoculation separately. This is to be regretted, but it is necessary, owing to a fire which burned the Agricultural Hall at Clemson College on April 2, 1925, and destroyed most of the notes concerning the organism which had been accumulated up to that time. Most of the early work must therefore be reported from memory and can not be given in detail.

The records show that there is more or less variation in susceptibility among different varieties of peas. The variety Telephone was by far the most susceptible of the three or four varieties used. It was observed also that young, succulent, rapidly growing tissues are more easily infected than tougher, more mature ones. The Telephone variety is rank in its habit of growth and its tissues are very tender and succulent when young. It is possible that the greater susceptibility of this variety observed here may be ascribed to this condition.

The circumstances, in the order of decreasing importance, which favored infection were wounds, rapidly growing succulent tissues, and high humidity (secured by moist chambers). The moist chambers were quite unimportant. In fact, it was found so easy to produce infections without them that most of the later work was done without coverings of any kind.

The presence of wounds was very important, however. There were some infections where no known wounds were present, but these were very few as compared with the number which developed following injuries. It is difficult to believe that an organism exhibiting the infection characteristics of this one could produce a severe epidemic in the absence of some wholesale source of mechanical injuries to the crop affected. In the inoculations carried out in 1924, much of the work was done in the writer's garden, and no accidental or natural infections were discovered. It is worthy of note in this connection that Sackett emphasized the importance of mechanical injuries as predisposing to the disease, even with a strain of bacteria capable of producing stomatal infections in great abundance.

The fact that the one seed inoculation test which we have been able to make failed to produce the disease would indicate that the bacteria are probably not seed-borne. It is recognized, of course, that one such test is insufficient to be considered as proof of the point.

The only plants other than garden peas which were inoculated were red and white clover. There was not the slightest evidence of infection in either case.

CULTURAL CHARACTERISTICS AND IDENTITY OF THE ORGANISM

The organism was studied on the following media: nutrient agar, nutrient gelatin, milk, litmus milk, potato plugs, nutrient broth, nitrate broth,

peptone broth, starch agar, dextrose broth, lactose broth, saccharose broth, and glycerin broth.

The behavior of the organism on these media, with exceptions noted below, was similar to that of Sackett's *Pseudomonas pisi*. Accordingly, an attempt was made to secure an authentic culture of that organism for purposes of comparison. Professor Sackett's type culture had been lost, however, and no other could be obtained. Under these circumstances Professor Sackett kindly examined the South Carolina culture, made the necessary tests, and reported that, in his opinion, the organism is the same as the species he described, although possibly a different strain. The most important difference he noted was the failure of the South Carolina strain to produce a curd in milk, while his produced a soft curd. He did not observe reduction of nitrate, although our tests indicate a weak action of this kind. In litmus milk he noted more marked color banding than was the case with the Colorado organism, but he does not consider this an important difference. Both organisms exhibited considerable variation in this regard. Our results indicate that the organism does not digest the casein, at least not quickly, as the addition of acid some months after inoculation and after the culture had become thoroughly blue threw down a curd, in addition to reddening the litmus.

A further difference in the two strains lies in the ease of securing infections. Sackett's strain produced stomatal infections with great ease. No such infections with the South Carolina organism have been noted.

All things considered, it seems best, at least for the present, to consider the organism as a strain of *Pseudomonas pisi* Sackett (*Phytomonas pisi* (Sackett) S. A. B.).[4] A culture is being deposited with the type culture collection at the McCormick Institute, Chicago, Illinois.

LONGEVITY OF THE ORGANISM IN CULTURE

During the summer of 1923 it became impossible to obtain transfers of the original culture 4; and the practice was adopted of transferring the remaining cultures—A, B, C, and D (see table 1)—at intervals of about two weeks. For more than a year the old cultures were not discarded, so that at the end of that time a large number were on hand, many of which had been thoroughly dried for months. They were carried on ordinary nutrient agar.

Late in June or early in July, 1924 (record of exact date burned), sterile nutrient broth was poured into each tube, and subcultures were made from those which clouded. The tubes so treated contained various transfers of

[4] Society of American Bacteriologists. Manual of determinative bacteriology. p. 181. Williams and Wilkins Co. 1923.

TABLE 1.—*The results of inoculating several varieties of garden peas and two species of Trifolium with Pseudomonas pisi Sackett in 1922–1925.*

Date	Test plant[1]	Parts inoculated	Source of inoculum	Manner of inoculation	Result	Notes
1922[2]	Garden pea	Pods and stems	Original isolations	Needle pricks; moist chambers used	−	
1923[2]	Garden pea, Tel. included	Pods	Original culture 4, some of the reisolations	Needle pricks; moist chambers used	+	Several infections, 4 reisolated strains, A, B, C, and D
1924[2]	Garden pea, about three varieties, including Tel.[3] Tel.[4]	All parts	Cultures A, B, C, and D	Spores rubbed or sprayed on uninjured plants and on plants pricked with a needle; with and without moist chambers	 +	Where infections occurred without pricking there were possibly other minute wounds
1924[2]	Tel., L.E., E.E.A.		Culture B2Y (reisolation of same season)		+	
March, 1925[2]	Tel., L.E., E.E.A.	Stems	Culture 4	Needle pricks; moist chambers used	−	
March, 1925[2]	Tel., L.E., E.E.A.	Stems	Culture B2Y	Needle pricks; moist chambers used	+	
March 26, 1925	E.E.A.	Pods	Culture 4	Needle pricks; moist chambers used	+	See plate 1
April 17, 1925	E.E.A.	Pods	Spot on pod from inoculation of March 26	Needle pricks; moist chambers used	+	See plate 2
April 24, 1925	E.E.A.	All parts	Spot on pod from inoculation of April 17	Needle pricks; no moist chambers used	 +	Leaves not affected
April 24, 1925	E.E.A.	Pods	Spot on pod from inoculation of April 17	Inoculum rubbed gently on pod	+	

TABLE 1.—*Continued.*

Date	Test plant[1]	Parts inoculated	Source of inoculation	Manner of inoculation	Result	Notes
April 29, 1925	E.E.A., Tel.	Stems	Spot on pod from inoculation of April 24	Needle pricks; no moist chambers used	+	More vigorous infection on young succulent parts and on Tel.
May 7, 1925	L.E., Tel.	All parts	Spot on pod from inoculation of April 24	Inoculum sprayed on uninjured plants; no moist chamber used	+	See plate 4
May 7, 1925	L.E., Tel.	Pods and stems	Spot on pod from inoculation of April 24	Needle pricks and spray; no moist chamber used	L.E. – Tel. +	See plate 5
May 7, 1925	L.E., Tel.	Leaves	Spot on pod from inoculation of April 24	Needle pricks and spray; no moist chamber used	L.E. – Tel. +	See plate 3
Feb.[2] or March, 1925	Tel., E.E.A., L.E.	Seeds	Culture 4 or B2Y	Seed soaked 15 minutes in bacterial suspension	–	
	Trifolium pratense L.	Stems and leaves		Needle pricks; moist chambers used	–	
	Trifolium hybridum L.	Stems and leaves		Needle pricks; moist chambers used	–	

[1] Varietal names of garden peas are abbreviated as follows: Tel. = Telephone; L.E. = Lightning Excelsior; E.E.A. = Extra Early Alaska.

[2] Records burned in the fire of April 2, 1925.

[3] Many of these inoculations were made in the writer's garden.

[4] Inoculation made in the writer's garden.

cultures A, B, C, and D, and the last few transfers of culture 4. The subcultures from the oldest ones which grew were put through physiological tests in comparison with the current transfers of A, B, C, and D. The earliest dated transfers, from which cultures were secured, which agreed in physiological tests with the regular cultures, were as follows:

June 1, 1923	4 and A	June 18, 1923	B, C, and 4
June 8, 1923	B	June 22, 1923	A and B
June 9, 1923	4		

Culture 4, recovered from transfer of June 1, 1923, was later (1925. See table 1) inoculated into pea pods and produced typical infection. This culture, therefore, was still viable and virulent after being dried out for several months and without transfer for over a year. Its total continuous life on artificial media was approximately three years.

DISTRIBUTION AND SERIOUSNESS OF THE DISEASE

The first record of this disease in South Carolina seems to have been the one from Latta in 1922. At that time it appeared to be merely an unimportant pod spot. Since then, however, it has taken the form of a stem blight which reduces the yield seriously. It was severe both in 1924 (see footnote 2) and in 1925.[5] It is also a serious factor in other parts of the country.

CONTROL

Two facts determined by this study have a bearing on the problem of control. First, the apparent necessity for wounds before abundant infections can take place indicates that methods of cultivation which reduce mechanical injuries to a minimum will help keep the infection down. Secondly, the difference noted in varietal susceptibility suggests that varieties which are sufficiently resistant to suffer little damage from the disease can be found or developed. The latter is probably the most promising line of attack on the control problem. This opinion is shared with Sackett, who based his conclusion on field observations of the susceptibility of several varieties of field peas.

SUMMARY

A pod spot of peas, discovered near Latta, South Carolina, in 1922, is caused by an organism believed to be a strain of *Pseudomonas pisi* Sackett. This bacterium, as shown by these studies, is an active wound parasite in pods, stems, and leaves of the garden pea, particularly when the tissues are young and succulent. Occasional infections without known wounds occur.

[5] Personal communication by Dr. W. D. Moore, assistant plant pathologist in extension work, Clemson College, S. C.

Under some circumstances it is able to live in culture without transfer for over a year, even without protection from drying. Some varieties of peas seem to be more resistant than others, and the most promising line of attack on the problem of control appears to be an attempt to find, or develop, suitable resistant or immune varieties.

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EXPLANATION OF PLATE XVI

1. Pod of Extra Early Alaska garden pea inoculated with culture 4. Inoculated March 29, 1925; photographed April 7.

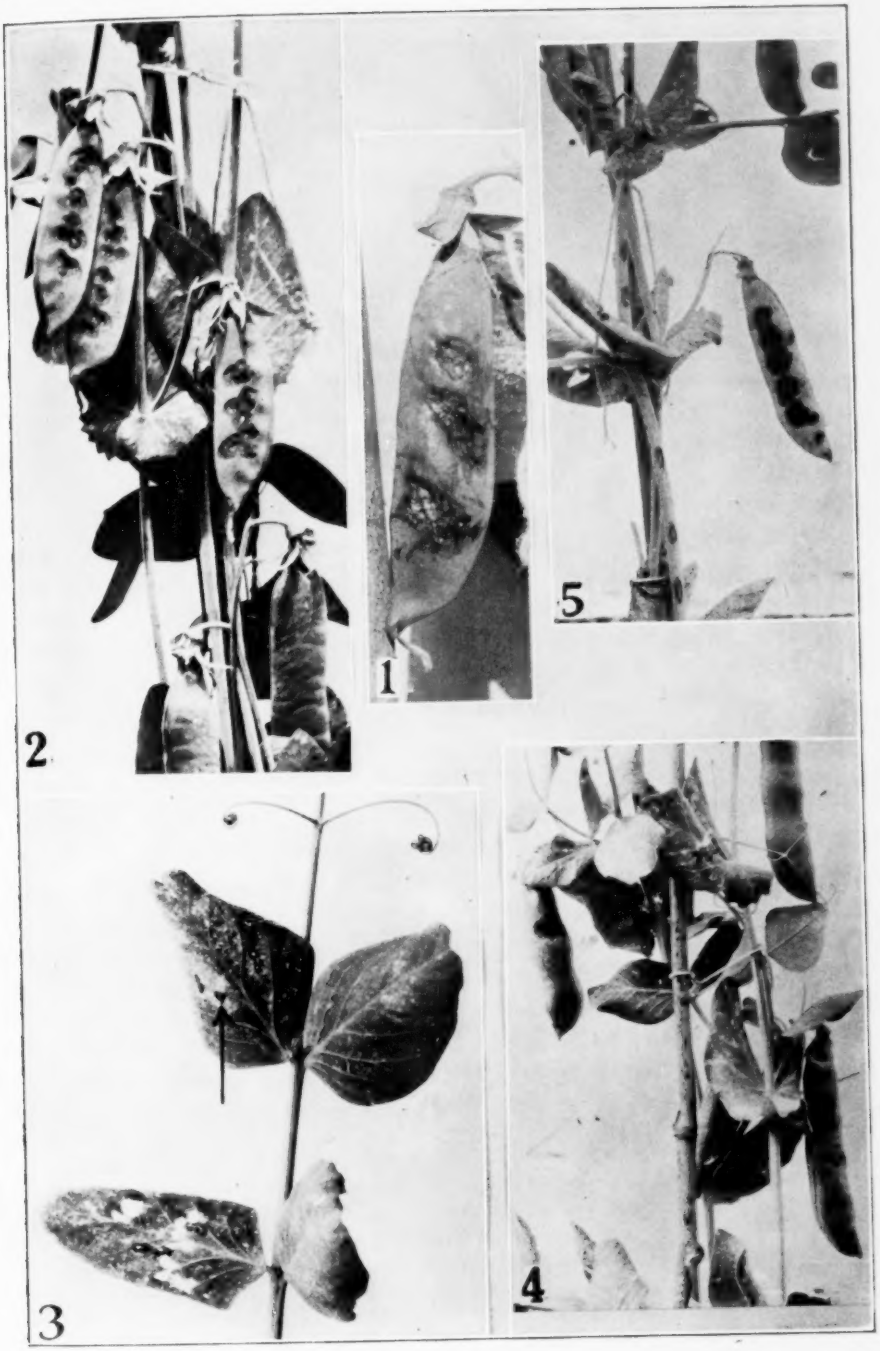
2. Pods of Extra Early Alaska pea inoculated April 17, 1925, from one of the spots on the pod shown in fig. 1. Photographed April 23.

3. Lesions on leaf of Telephone pea. Inoculated May 7, 1925; photographed June 1. The arrow points to one of the lesions.

4. Spots on pods of Telephone pea produced by spraying the inoculum onto the plants without wounding. Inoculated May 7, 1925; photographed May 19.

5. Stem and pod lesions on Telephone pea produced by spraying the inoculum onto the wounded plants. Inoculated May 7, 1925; photographed June 1. The pod in this case is nearly ripe.

Note: There was a severe infestation of red spider in the greenhouse while the inculation experiments were in progress, and the grayish appearance of the leaves in the figures is due to injury from this source.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FACTORS AFFECTING THE DEVELOPMENT OF FLAX RUST, MELAMPSORA LINI (PERS.) LEV.

HELEN HART[1]

Rust, caused by *Melampsora lini*, is very injurious to fiber flaxes, often ruining the fiber. Seed flax does not appear to be severely injured except in epidemics, which are not uncommon in the flax-growing states. Barker (24) observed that seed flax often was stunted or killed outright when young plants were heavily infected. But older plants also may be very severely injured. The writer saw plants of N. D. R. 114 from Meeker County, Minnesota, in 1925 which were quite ruined by the rust. The infection on the pedicels was so heavy that the seed was badly shriveled and the yield very low. The disease seems most destructive on low-lying soils, and is especially injurious in the Red River Valley of North Dakota and Minnesota. Brentzel (23) reported that local losses in the Red River Valley in 1919 ranged from a trace to 100 per cent. The average annual loss for North Dakota is estimated at one per cent of the entire crop. In Minnesota the disease occurs quite generally throughout the State, and causes an annual loss of something less than one per cent (24).

REVIEW OF PREVIOUS INVESTIGATIONS

In 1801, Persoon (13) described a rust collected on *Linum usitatissimum* L. and *L. catharticum* L. and named it *Uredo miniata* var. *lini*. It was not until 1847 that Léveillé (7) placed the fungus in the genus *Melampsora* under the name *Melampsora lini*. Although flax rust has been known so long, it has been but little investigated until recent years. Körnicke, in 1865, probably was the first of the earlier workers to suggest a possibility of specialization within the species. Since 1910, Palm (12) and Bucheim (4) have investigated this phase of the subject and have concluded that the rust is not the same on all species of *Linum*. Arthur (2) showed that *Melampsora lini* is eu-autoecious, but he did not find it specialized for a

[1] Cooperative investigations between the U. S. Department of Agriculture, Bureau of Plant Industry, Office of Cereal Investigations, and the Minnesota Agricultural Experiment Station. Published with the approval of the Director as paper No. 586 of the Journal Series of the Minnesota Agricultural Experiment Station.

The writer wishes to acknowledge her indebtedness to Doctor E. C. Stakman for invaluable assistance throughout the investigation.

particular species of *Linum*. The cytology and sexual fusions of *M. lini* have been described by Fromme (6), while Tobler (21) recently studied the histological effects of the pathogene upon the host tissues. In Ireland, Pethybridge and coworkers (14, 15, 16) have been investigating the general aspect of flax rust since 1920.

OBJECTS OF THE PRESENT INVESTIGATION

The investigation was undertaken to determine: (1) the factors affecting infection, (2) the effect of environmental factors on the development of rust subsequent to infection, (3) the manner of entrance of the uredinial germ tubes into susceptible and resistant hosts, (4) the histological relations between pathogene and host, (5) the possible occurrence of physiologic forms.

FACTORS AFFECTING THE GERMINATION OF SPORES

Aeciospores. Aeciospores germinate readily in distilled water, each spore sending out from one to five short, thick germ tubes. Under favorable conditions, one or two of the germ tubes develop faster than the rest, so that the growth is concentrated in one or two long infection threads. Under less favorable conditions, the germ tubes develop simultaneously and none of them become very long. Under optimum conditions, aeciospores begin to germinate within 45 minutes, and within 1½ hours they often have produced germ tubes 20 microns long.

Aeciospores do not germinate in a saturated atmosphere, but do so readily when floated on distilled water. It is well known that the spores of certain fungi germinate more readily when host tissue is added to water, some stimulatory substance apparently being supplied by the host. When crushed flax tissue was added to distilled water, the percentage of spore germination increased very slightly. Host tissue apparently does not greatly stimulate the germination of aeciospores.

Aeciospores germinate within a wide range of temperature, the minimum lying close to 0° C., while the maximum is between 26° and 27° C. The optimum is about 18° C., at which temperature the highest percentage of spores germinate and produce the most vigorous germ tubes. Those formed at the higher and lower temperatures are weaker and grow more slowly (table 1).

Urediniospores. Each germinating urediniospore sends out one or more germ tubes from its equatorial germ pores. The thin-walled, reddish-orange tubes usually are branched, and, as in the case of aeciospores, one, or sometimes two, grow faster and more vigorously than the rest (Fig. 1, C). Under optimum conditions, the small, knob-like germ tubes push out through the pores within one and one-half hours (Fig. 1, A). Under optimum con-

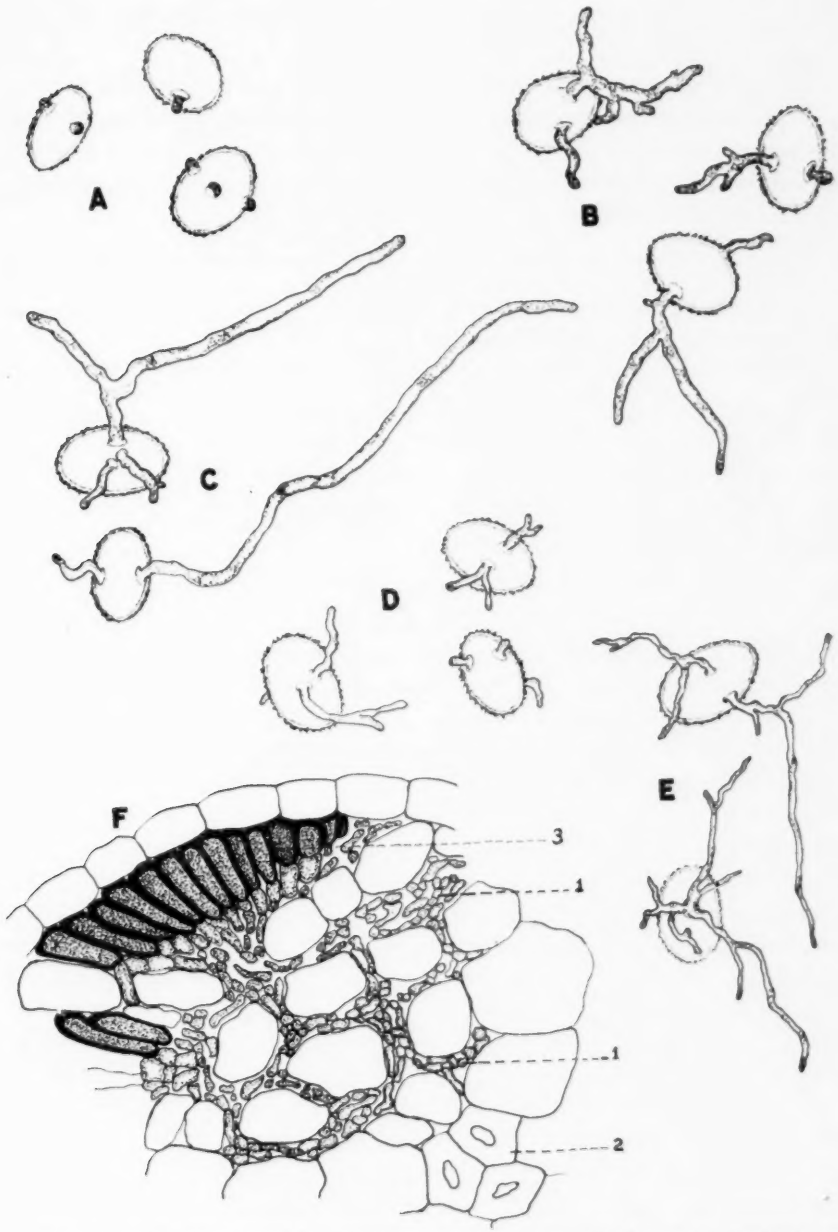

FIG. 1. A–E. Germinating urediniospores. A. After 1½ hours in distilled water at 18° C. B. After 4 hours in distilled water at 18° C. C. After 9 hours in distilled water at 18° C. D. After 24 hours at 2.5° C. E. After 48 hours at 25° C. F. Cross section of rusted stem of Winona, showing the intercellular hyphae (1) which have separated the cortical cells of the host and have penetrated as far as the bast fibers (2). A layer of young teliospores (3) has been formed just beneath the epidermis.

ditions, some of the germ tubes reach a length of 20 microns within 3 hours, and after 24 hours many are 150 microns long.

Contact with water is necessary for the germination of urediniospores. The writer never has observed their germination in a saturated atmosphere, while those floating on distilled water germinated readily. As in the case of aeciospores, the addition of host tissue to distilled water did not stimulate germination appreciably.

TABLE 1.—*The effect of temperature on the germination of aeciospores of Melampsora lini in distilled water*

Temperature in degrees C.	Percentage of germination	Character of germ tubes
0– 2	2.5	Very short and slender; weak
6– 7	46.5	Short, but fairly vigorous
11–12	81.5	Long and vigorous
17–18	90	Long and very vigorous
21–22	80	Long and vigorous
24–25.5	62	Short and very weak
28–29	0	

Oxygen is required for urediniospore germination. Spores floating on distilled water germinate readily, while those submerged in water, and therefore having a diminished supply of free oxygen, seldom germinate. From 60 to 80 per cent of spores germinated when abundant oxygen was available, while none germinated when all of the oxygen was removed by pyrogallol.

Temperature has a decided effect on the germination of urediniospores. The optimum is about 18° C., the same as for aeciospore germination. The highest percentage of spores germinate at the optimum, but fairly abundant germination and vigorous germ tube growth is obtained at temperatures ranging from 6° to 23° C. Beyond these limits the percentage of germination decreases, and germ tubes are short and weak (Fig. 1, D and E). Even at 23° C. the germ tubes are poorly developed. At 27.5° C. the spores did not germinate, the maximum temperature apparently being about 26° or 27° C. The minimum lies very close to 0° C., for a small percentage of spores germinate at from 0° to 1° C. In one instance, 9 per cent of the spores germinated during eight hours at from 0.5° to 2.5° C., but the germ tubes grow very slowly at such low temperatures (Fig. 1, D). Table 2 gives the percentages of urediniospore germination obtained at different temperatures in five representative trials.

Light seems to have little or no effect on the germination of the urediniospores of *M. lini*. Germination tests were made in light and darkness at

TABLE 2.—*The effect of temperature on germination of urediniospores of Melampsora lini in distilled water*

Series number	Temperature in degrees Centigrade								
	0.5–2.5	6.7	11–12	15–17	18–19	21–23	24.5–25.5	27.5	30
	Percentage of germination[a]								
1	–	37	38	45	–	54	–	0	0
2	–	62	65	78	87	50	16	0	–
3	–	41	55	47	–	56	9	–	0
4	9	60	75	–	87	73	6	0	–
5	tr	77	85	86	91	15	4	0	–
Average	5	55	64	64	88	50	9	0	0

[a] tr = less than 1%; – = no test made.

temperatures of 14°, 17°, and 23.5° C. In every case the development of the germ tubes and the germination percentages at a given temperature were approximately the same in light and darkness. Table 3 gives the averages obtained during three trials.

TABLE 3.—*The effect of light on the germination of urediniospores of Melampsora lini in distilled water at different temperatures*

Temperature in degrees C.	Light conditions	Percentage of germination
14	Artificial electric light	42
14	Darkness	45
17	Sunlight	62
17	Darkness	60
23.5	Sunlight	4
23.5	Darkness	5

Teliospores. Pethybridge, Lafferty, and Rhynehart (15) recently have described the germination of teliospores. Each spore produces a short, thick promycelium, usually divided into four cells by transverse walls. A single oval or pear-shaped basidiospore is borne on the sterigma of each cell.

Teliospores require a rest period before germination. The writer made several attempts to shorten the period of dormancy, but failed in all of them. Teliospores stored out-of-doors during the winter did not begin to germinate until early June. In 1924, teliospores began to germinate on June 12, and flax rust was first found in the field on June 20.

Thiel and Weiss (20) found that the rest period of teliospores of *Puccinia graminis tritici* (Pers.) Erikss. and Henn. could be shortened by treat-

ment with one per cent citric acid for fifteen minutes. The writer soaked teliospores of *Melampsora lini* in one per cent citric acid for periods varying from ten to thirty minutes, but the rest period was not shortened.

Noble (11) found presoaking necessary to the germination of spores of *Urocystis tritici* Koern. Teliospores of *Melampsora lini* were presoaked in distilled water for periods varying from a few hours to a week, at the end of which time flax tissue was added, but none of the spores germinated.

Alternate freezing and thawing did not shorten the period of dormancy, nor did lowering the surface tension by films of benzaldehyde, citral, oil of bergamot and oil of garlic have any effect. Exposure to ether vapor for periods varying from two seconds to two minutes also was ineffective. Solutions of sodium nitrate, calcium nitrate, and potassium nitrate were used in concentrations of N/1, N/10, N/25, and N/300, but none of the spores germinated. If the dormancy period can be shortened, the right methods for accomplishing it have not been found. As the methods which are effective in stimulating germination of spores of several other fungi were ineffective for teliospores of *M. lini*, it is possible that a period of real "after ripening" is necessary.

VITALITY OF SPORES

As the rust is spread largely by urediniospores, it is important to know how long they retain their viability.

Temperature and relative humidity are known to affect the vitality of spores. The effect of these two factors on the vitality of urediniospores was therefore investigated. Uredinial material from greenhouse cultures was kept at 7° C., 11° C., 17° C., and 22° C., in relative humidities of 0, 20, 40, 60, and 80 per cent. There was a plus and minus fluctuation of about one degree for the different temperatures. The relative humidity was controlled by using various concentrations of sulphuric acid. The material was suspended above the acid in sealed bottles kept at different temperatures. The results of the germination tests are given in table 4.

Seventy per cent of the spores germinated at the beginning of the experiment. As time passed, the germination percentages gradually decreased until none of the spores germinated. It will be seen from table 4 that the spores lost their vitality more rapidly at the higher temperatures than at the lower ones, and at the extreme relative humidities than at the intermediate ones. At the end of five weeks nearly all the spores kept at 22° C. had lost their viability, for less than one per cent of those kept at a relative humidity of 40 per cent germinated, and none of those kept at the other relative humidities germinated. After eleven weeks, all spores, except those kept at 7° C. and 60 per cent relative humidity, had lost their viability. After twelve weeks none of the spores germinated, although it is possible

that the urediniospores remain viable longer at still lower temperatures. However, this seems improbable; and it seems unlikely, from the results obtained, that the urediniospores retain their viability during the winter.

TABLE 4.—*The effect of temperature and humidity on the viability of urediniospores of Melampsora lini*

Temperature in degrees C.	Relative humidity in per cent	Time in weeks					
		1	3	5	7	11	12
		Percentage of germination in distilled water[a]					
7	0	tr	0	0	0	0	0
	20	38	11	7	2	0	0
	40	18	9	11	8	—	—
	60	52	6.5	16	12.5	tr	0
	80	40	7.5	10	2.5	0	0
11	0	2	1.2	tr	0	0	0
	20	42	10	3	1.5	0	0
	40	37.5	25	4	2	0	0
	60	38	tr	2	tr	0	0
	80	36	0	0	0	0	0
17	0	5	0	0	0	0	0
	20	25	9	5	tr	0	0
	40	24.5	3	tr	tr	0	0
	60	38	10	tr	0	0	0
	80	tr	0	0	0	0	0
22	0	—	—	—	—	—	—
	20	32	tr	0	0	0	—
	40	39	2	tr	0	0	—
	60	11.5	tr	0	0	0	—
	80	tr	0	0	0	0	—

[a] tr = less than 1%; — = no test made.

As is shown in table 4, the decrease in the percentage of spore germination is more gradual at low than at high temperatures. The largest number of spores seemed to remain viable for the longest period of time at 7° C. The most favorable relative humidity seemed to be 40 per cent in most cases, although at 7° C. a relative humidity of 60 per cent seemed most favorable.

INFECTION PHENOMENA

The uredinial germ tubes enter the host through the stomata. The manner of entrance is similar to that described by Stakman (18) for *Puc-*

cinia graminis tritici. The urediniospores of *M. lini* produce germ tubes which grow toward the stomata, following very closely the contour of the epidermis. The tube swells directly over the stomatal opening and the protoplasmic contents are concentrated in an appressorium (Fig. 2, B). A slender penetration tube then passes between the guard cells and forms a substomatal vesicle. Fig. 2, B, D, and E, illustrate the entrance of the pathogene in a susceptible host. From the substomatal vesicle numerous infection threads grow out in all directions, branching between the cells of the host and sending haustoria into them (Fig. 2, E). The invaded host cells appear to be uninjured for a considerable time after infection.

Miss Marryat (8) found that the uredinial germ tubes of *Puccinia glumarum* (Schum.) E. and H. entered highly resistant hosts as well as susceptible ones. Stakman (18), Newton (10), and Allen (1) found the same to be true of *Puccinia graminis tritici* (Pers.) E. and H. Flax rust is very similar, for the uredinial germ tubes enter the stomata of Argentine flax, a practically immune variety. There is no externally visible sign of entry, but it is evident from histological study that typical appressoria, penetration tubes, and substomatal vesicles are formed (Fig. 2, C). Infection threads grow out from the substomatal vesicle, and haustoria sometimes may be sent into the cells. The host cells seem extremely sensitive to the rust hyphae, and, within a week after inoculation, some of the host cells are killed by the fungus which is unable to establish itself and finally dies (Fig. 2, F). The action is usually very rapid, for only a few of the host cells are killed by the pathogene before it dies.

The time required for the entrance of the pathogene into the host was determined by varying the time during which inoculated plants were kept in the moist chamber. Fourteen pots of flax, of ten plants each, were inoculated and placed in the moist chamber at the same time. They were removed in pairs after intervals of 1½, 3, 6, 9, 14, 24, and 48 hours, respectively. Observations were made 10 days after inoculation. No uredinia appeared on plants incubated only 1½ hours. Even under optimum conditions, the urediniospores did not begin to germinate until after 1½ hours, so that no germ tubes were long enough to effect entrance when plants were kept in a moist chamber for only this length of time. The plants incubated for three hours became very slightly infected, 11 uredinia appearing on a total of 20 plants.

The germ tubes are often 20 microns long at the end of 3 hours, so that entrance may be effected very easily within that time if a spore is near a stoma on the leaf. Light infection developed on plants incubated in the moist chamber for 6 hours, and moderately heavy infection on those plants incubated 9 hours and 14 hours. Plants kept in the moist chamber for 24

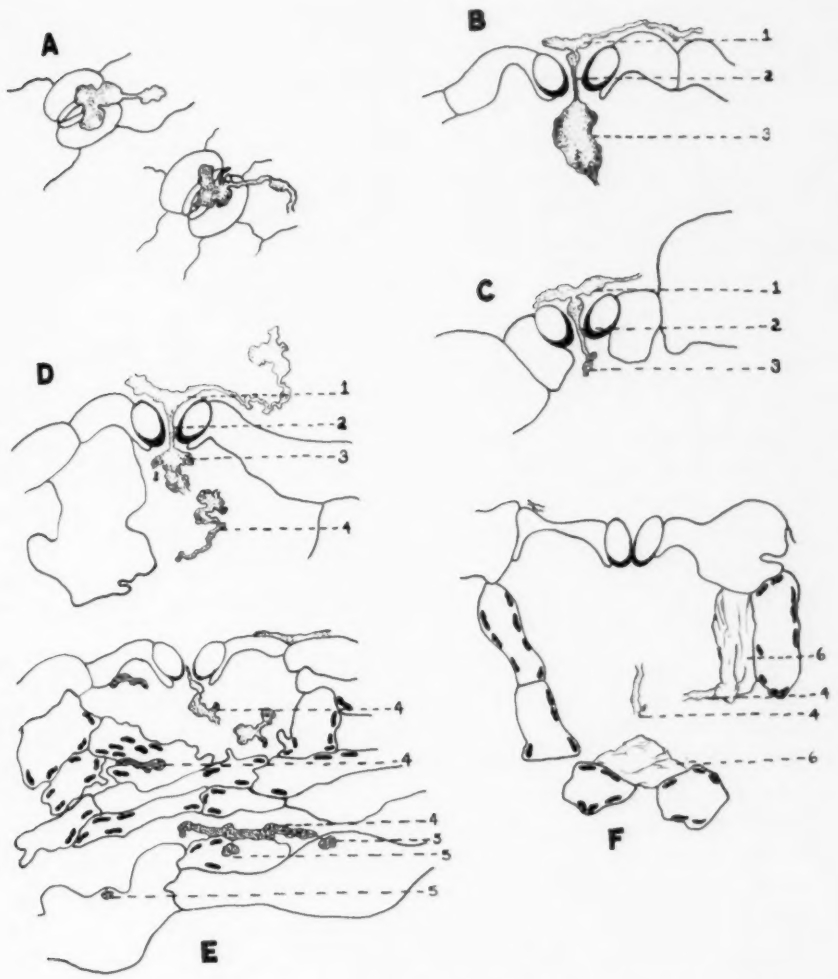

FIG. 2. A. Surface view of two appressoria formed by uredinial germ tubes over stomata. After forty-eight hours in the moist chamber. B. Section of a leaf of Winona flax, taken after forty-eight hours in the moist chamber, showing an appressorium (1) over the stoma, a slender penetration tube (2) passing through the stomatal opening and a sub-stomatal vesicle (3) formed beneath the stoma. C. Section of a leaf of Argentine flax, taken after forty-eight hours in a moist chamber, showing a typical appressorium (1), penetration tube (2) and small sub-stomatal vesicle (3). D. Section of Winona leaf, taken four days after inoculation, showing an appressorium (1), penetration tube (2), sub-stomatal vesicle (3) and the hyphae (4) developing from the sub-stomatal vesicle. E. Section of Winona leaf, taken five days after inoculation, showing the intercellular hyphae (4) which penetrate the host tissue and send haustoria (5) into the host cells. F. Section of Argentine leaf, taken seven days after inoculation, showing infection threads (4) which have destroyed certain host cells (6).

and 48 hours became heavily infected. The fact that germ tubes may enter in as short a time as 3 hours indicates that a small amount of moisture for a relatively short time may be sufficient for the process in the field.

FACTORS AFFECTING LENGTH OF INCUBATION PERIOD

In the greenhouse, at temperatures between 16° and 23° C., uredinia appear 9 or 10 days after inoculation. The first evidence of infection is the appearance of flecks, minute circular areas lighter in color than that of the normal, green leaf. The light areas soon take on an orange color, owing to the formation of urediniospores beneath the epidermis of the host. Within 24 hours the epidermis is ruptured by the fully formed uredinium.

The length of the incubation period is greatly influenced by external conditions. The effect of environment on the incubation period of the uredinial stage was studied most extensively. It was found that environmental conditions immediately following inoculation affect the germination of spores and the entrance of the pathogene, and, after the fungus enters its host, environmental conditions are important in determining the rapidity of development and the severity of the infection. Factors influencing spore germination may be relatively unimportant in uredinial formation and *vice versa*.

Moisture. As shown above, no rust develops on plants supplied with abundant moisture for only 1½ hours. Abundant moisture for 3 hours, immediately after inoculation, is sufficient to enable a few spores to germinate and send germ tubes into the host, but severe infection is obtained only when the time is extended to 24 hours.

Light. Light has virtually no effect on the germination of the spores of *M. lini*, and evidently has no influence on the entrance of the germ tubes. One series of plants was inoculated with urediniospores and placed in a moist chamber exposed to sunlight during the day and to artificial light during the night. A second series, inoculated at the same time, was placed in a moist chamber from which all light was excluded by a cover of six layers of heavy wrapping paper. The temperature was favorable to spore germination, varying from 17° C. to 23° C. After 48 hours, the plants of both series were removed from the moist chambers and placed on the greenhouse bench. On the 11th day uredinia appeared in both series, and the degree of infection was approximately the same, indicating that the germ tubes are able to enter the host equally well in darkness and in light.

Light appears to have little effect on germination of the spores and subsequent entrance of the germ tube into the host, but it is essential to pustule formation after the pathogene has established itself within the host. On plants exposed to continuous electric light with an intensity of 400 to 600

foot candles at the leaf surface (a low intensity when compared with that of sunlight), uredinia appeared 6½ days after inoculation, while check plants, under normal conditions of alternate sunlight and darkness, did not fleck until the 9th day after inoculation. While increased light shortens the incubation period, decreased light tends to lengthen it, and retards the formation of uredinia. On plants shaded with black cambric and placed in a dark corner of the greenhouse, uredinia did not develop until the 14th day after inoculation, 5 days later than on the check plants exposed to full sunlight, and almost 8 days later than on plants exposed to continuous electric light.

Temperature. The influence of temperature during the moist chamber period was determined by placing inoculated plants in moist chambers kept at different temperatures. All the plants were inoculated under uniform conditions and one-third of the lot placed in a moist chamber at 4–10° C., one-third at 16–20° C., and the remaining third at 27–30° C. After 48 hours, all were removed to the greenhouse bench and kept at a temperature of 16–22° C. under optimum light conditions. The first uredinia appeared 9½ days after inoculation, on the plants which had been kept in the moist chamber at 4–10° C. Ten days after inoculation, uredinia appeared on the plants incubated in the moist chamber at 16–20° C. None of the plants in the third series became infected, the temperature being too high for the germination of spores. The results of the experiment agree with the results of spore germination tests, for the urediniospores germinate readily at temperatures between 6° and 22° C., while the maximum temperature for germination is about 26° or 27° C.

Uredinia may form within a fairly wide range of temperatures, but the optimum coincides with the optimum temperature for spore germination and that of the growth of the flax plant. Nine pots of flax were inoculated and placed in a moist chamber at from 16° to 20° C. After 48 hours, they were subjected to different conditions. Three pots were exposed to temperatures of from 7° C. to 14° C., three were placed at 16° to 22° C., and three at 25° to 30° C. The results are summarized in table 5. The lower and higher temperatures retard the formation of uredinia. At 16° to 22° C., uredinia first appeared on the 10th day after inoculation. On the 11th day, the flecks and uredinia were numerous, and from 65 per cent to 100 per cent of the plants were infected. On the 14th day the rust infection was severe, the uredinia being large and very numerous. At the lower temperatures, 7–14° C., the first uredinium appeared 14 days after inoculation, and at the higher temperatures, 25–30° C., uredinia appeared on the 15th day. Infection was always light at the lower and higher temperatures; the uredinia were always small and relatively few in number.

TABLE 5.—*The effect of temperature on the development of uredinia of Melampsora lini on Winona flax, C. I. 179, inoculated with urediniospores and kept in a moist chamber for 48 hours at 16°–20° C.*

Temperature during period of incubation		Series no.	Number of days after inoculation and degree of infection							
Moist chamber	After removal		10	11	12	13	14	15	16	17
	7–14° C.	1								1 uredinium
		2						Cotyledons of 1 plant heavily infected	Numerous uredinia and flecks	
		3							2 small uredinia	Very light
16–20° C.	16–20° C.	1	2 uredinia, no flecks	11 uredinia, numerous flecks, 66% plants infected	Light		Moderate	Moderate	Heavy	Heavy
		2	2 uredinia, numerous flecks	Uredinia and flecks numerous. 100% plants infected	Moderate	Moderate	Heavy	Heavy	Heavy	Heavy
		3	1 uredinium, few flecks	Uredinia and flecks numerous. 80% plants infected	Moderate	Moderate	Heavy	Heavy	Heavy	Heavy
	26–30° C.	1								1 uredinium
		2					1 uredinium	1 uredinium, flecks	Light	Light
		3						1 uredinium		Very light

THE EFFECTS OF SOIL NUTRIENTS ON RUST INFECTION

Soil nutrients influence the growth and vigor of the host plant and thus indirectly influence the development of the rust. Obligate parasites, such as *M. lini,* usually thrive best on healthy plants. In order to ascertain the effect of nutrients, Winona flax, C. I. 179, was planted in sterilized soil and supplied with nutrient solutions of calcium acid phosphate, magnesium acid phosphate, potassium nitrate, sodium nitrate, and potassium sulphate made up in the following proportions:

2.944 gm. $CaHPO_4$	1000 c.c. distilled H_2O
2.980 gm. $MgHPO_4$	do
1.728 gm. KNO_3	do
1.452 gm. $NaNO_3$	do
2.980 gm. K_2SO_4	do

Check plants were supplied with distilled water.

Inoculations were made six weeks after planting. At that time, the plants in the acid phosphate series were larger and more vigorous than the others. The leaves were large and bright green, and nearly every plant had developed several branches. Plants in the nitrate series grew moderately well, developing strong, erect stems which seldom branched. The leaves were smaller and darker in color than those in the phosphate series. Plants supplied with sulphate grew very slowly. The leaves were very small, and the plants were about half as high as those in the phosphate or nitrate series. Even the check plants appeared more vigorous than those supplied with sulphate.

The plants were inoculated with urediniospores of *M. lini,* kept in a moist chamber for 48 hours, and then removed to the greenhouse bench. Observations were made on the 10th day after inoculation and are given below.

Phosphate series: Uredinia numerous, large, and fully formed, with the ruptured epidermis of the host evident. Slightly greater infection on plants in the $MgHPO_4$ series than those in the $CaHPO_4$ series.

Nitrate series: Uredinia numerous but not large; some uredinia fully formed, while others were just rupturing the epidermis of the host. Somewhat greater infection on the $NaNO_3$ series than the KNO_3 series.

Sulphate series: Uredinia few and very small; just breaking the epidermis of the host.

Check plants: Uredinia few and small.

Uredinia seem to appear sooner, and in greater abundance, on the most vigorous plants, a result which is to be expected. Pethybridge, Lafferty and Rhynehart (15) state that the intensity of a flax rust attack appears to increase with the luxuriance of host growth. Observations were again

made 17 days after inoculation, and those plants furnished with phosphates and with nitrates were very severely infected. There was very light infection, and practically no injury, on plants in the sulphate series and in the distilled water series. The plants in the phosphate series always were most severely infected.

HISTOLOGICAL RELATIONS BETWEEN HOST AND PATHOGENE

Rust attacks all the above-ground parts of the flax plant: stems, cotyledons, leaves, pedicels, and sepals. Early in the season, when the uredinial stage is prevalent, the rusted leaves are most conspicuous, but later, after the development of teliospores, the rust appears to be most abundant on the stems. It is the stems of fiber flax which are damaged, a moderate amount of rust causing a great deal of injury. The rust on the leaves is just as injurious to seed flax as that on the stems; the leaves often are killed and the entire plant may be stunted, so that it yields poorly. Heavy infection causes the stems to become so brittle as to break at the point of attack.

The hyphae are intercellular, pushing their way between the host cells. Very often the mycelium is so abundant that the cells of the host are wedged apart and separated from each other (Fig. 1, F).

In all of the sections of infected flax stems examined, the mycelium seemed to be restricted to the cortical region. None of the hyphae were ever seen penetrating the woody portion of the stem. In most of the sections they had penetrated as far as the bast fibers, which are grouped in strands in the cortex. The fibers themselves are often surrounded by the mycelium. Tobler (21) has described the effect of the fungus on the bast fibers and states that often they are more severely attacked than any of the other tissues. He found that in some cases the pathogene seems to modify the wall substance so that the thick walls of the fibers become much thinner. When the rust is severe, the fibers collapse and shrivel as though the cells of the adjoining cortex had exerted a pressure upon them. Tobler states that in some cases the fibers appear to be lignified when attacked by the rust. Also the pathogene seems to have a preservative effect, protecting the tissues from decay, so that the fiber cannot be separated in the retting process which fiber flax must undergo.

Fiber flax undoubtedly is severely injured by the rust. Seed flax suffers little damage until the mycelium becomes abundant enough to kill the tissues or retard the growth of the plant. The attack on the stems causes them to become brittle and break easily, a condition which may result in a poor stand and decreased yield. The destruction of the fibers in seed flax is not of such direct importance as in fiber flax.

PHYSIOLOGIC SPECIALIZATION

Persoon (13, p. 216), in 1801, described a rust of flax collected on *Linum usitatissimum* L. and *L. catharticum* L., in which the uredinial stage was noted and designated as *Uredo miniata* var. *lini* Pers. Since that time, several authors have described flax rust, nearly every one placing the causal organism in a different genus. It has been described as *Uredo*, *Podosporium*, *Lecythea*, *Xyloma*, and *Podocystis*, but it was not until 1847 that Léveillé (7) placed the fungus in the genus *Melampsora*.

According to Saccardo (17), *M. lini* occurs on the following species of *Linum: L. catharticum* L., *L. usitatissimum* L., *L. narbonense* L., *L. nodiflorum* L., *L. marginale* A. Cunn., *L. alpinum* Jacq., *L. perenne* L., *L. strictum* L., and *L. austriacum* L.

Sydow (19) reports the occurrence of rust on these additional species of *Linum: L. anatolicum L. breweri*, *L. capitatum*, *L. congestum*, *L. corymbulosum*, *L. drymarioides*, *L. gallicum*, *L. hirsutum*, *L. lewisii*, *L. pubescens*, *L. possariodes*, *L. rigidum*, *L. sulcatum*, and *L. viscosum*.[2]

In the United States, Arthur (3) reports rust on *L. breweri* A. Gray, *L. drymarioides* Curran, *L. rigidum* Pursh., and *L. sulcatum* Redd.

Körnicke probably was the first to suggest the possibility of specialization within *Melampsora lini*. According to Bucheim (4), Körnicke pointed out, as early as 1865, that in Prussia the rust on *Linum usitatissimum* was a special variety which he named *M. lini* var. *liniperda*. Körnicke also pointed out that *Linum usitatissimum* often was free from rust under conditions where *Linum catharticum* rusted commonly.

In 1910, Palm (12) found that *M. lini* on *Linum usitatissimum* had much larger teliospores than those described for *M. lini* on *L. catharticum*. His attempts to infect *L. catharticum* with urediniospores from *L. usitatissimum* were unsuccessful. His results led him to regard the form on common flax as a distinct species, and he named it *Melampsora liniperda* (Körnicke) Palm.

In 1915, Eriksson (5) concluded that there were two forms of *M. lini*, one on common flax, *Linum usitatissimum*, and one on *L. catharticum*.

In 1915, Bucheim (4) made cross inoculations with urediniospores of *M. lini* on four different species of *Linum; viz.*: *L. catharticum*, *L. alpinum*, *L. strictum*, and *L. tenuifolium*. The results indicated that the rust on each of the four hosts was highly specialized, infecting its own particular host but being unable to infect other species of *Linum*.

Pethybridge, Lafferty, and Rhynehart (16), in 1922, failed to infect common flax with rust obtained from purging flax, *L. catharticum*.

Wild flax, *L. lewisii*, is common in the Dakotas and Montana, and is frequently rusted. If the forms of *M. lini* on the common flax, *L. usitatis-*

[2] The authors of these species are not given by Sydow.

simum, and the wild flax, *L. lewisii,* were the same, the wild flax might be important in spreading rust to the cultivated flax. Arthur (2) states that teliospores of *M. lini* from common flax infected *L. lewisii,* an indication that the rust is not specialized to a particular species in this country.

The writer attempted to ascertain to what extent physiological specialization occurs in *M. lini.* Winona flax, susceptible to the rust of common flax, was inoculated with urediniospores taken from the wild flax, *L. lewisii.*[3] No infection resulted on two lots of plants inoculated in the first series, but four small uredinia appeared on plants in the other two lots. At first it was thought that a successful transfer had been accomplished, but in view of the later experiments it is probable that accidental infection with urediniospores of rust from common flax had taken place, inasmuch as the plants were not as completely isolated as in later experiments. All subsequent attempts to cause infection were unsuccessful. It appears, therefore, that the rust on *L. lewisii* is specialized for its host and does not attack Winona, a susceptible variety of common flax.

In addition to Winona, the resistant Argentine was inoculated with urediniospores from *L. lewisii.* No infection resulted in any case. While Bucheim showed that the rust of common flax does not attack *L. catharticum, L. alpinum, L. strictum,* nor *L. tenuifolium,* in Europe, no reports other than Arthur's (2) have been made regarding its ability to attack the species common in the flax-growing areas of the United States. Therefore attempts were made to transfer the rust from common flax to *L. lewisii* and *L. rigidum,*[4] the two wild species which are probably most common in the western areas. Seed of *L. sulcatum,* a species common in Minnesota and Wisconsin, was collected, but it germinated so poorly that plants for only two inoculations were obtained. Infection did not occur in either case. The *L. lewisii* used in the experiment came from some plants collected the previous fall. New shoots put out by the perennial crown were grown in the greenhouse for three months. No rust developed on them, thus indicating probable freedom from previous infection. When *L. lewisii* was inoculated with urediniospores from common flax, the results were always negative. Only three trials could be made with *L. rigidum.* At the time of inoculation the plants were very small, there being only three or four leaves on each one. No infection was secured in the first trial, but in the second and third trials *L. rigidum* was severely infected with rust from common flax. The incubation period was somewhat longer than it usually

[3] Specimens of rusted *L. lewisii* sent by Professor E. B. Payson and by Mr. R. U. Cotter, of the University of Wyoming.

[4] Plants of *L. lewisii* were obtained from Mrs. F. M. Heath, through Professor Baird, University of North Dakota. Seed of *L. rigidum* was furnished by Professor E. J. Petry, State Agricultural College, Brookings, South Dakota.

is on common flax, as the uredinia did not develop until the 15th day after inoculation. After 20 days, the plants were severely rusted.

Apparently, *L. lewisii* need not be regarded as a source of infection of cultivated flax, for the rusts on the two species seem to be highly specialized; on the other hand, *L. rigidum* may prove dangerous, for the rust on common flax attacks this wild species. It was impossible to obtain naturally rusted *L. rigidum*, but it is quite probable that the rust can be transferred from this wild species to cultivated flax.

According to our present knowledge, the variety Argentine is virtually immune from rust, but it is possible that there is some form which can attack it. Rust on common flax, therefore, was collected from several different localities and cultured in the greenhouse. The following strains were maintained successfully: (1) from Purgatory Plats, Hennepin County, Minn.; (2) from University Farm, St. Paul, Minn.; (3) from Fargo, N. D.; (4) from Mandan, N. D.; (5) from Indian Head, Saskatchewan, Canada; (6) from Saskatoon, Sask., Canada. Argentine and Winona were inoculated with urediniospores from each of the rust collections. Host plants of various ages were used in order to determine whether the age of the host had any effect on its resistance. Plants of various ages, up to maturity, were inoculated. Winona became infected with every culture, the degree of infection ranging from moderate to heavy, but not varying much according to the age of the host. In most cases where Argentine was inoculated, the results were entirely negative, no signs of infection appearing. But in one experiment, two small flecks appeared on one plant out of six which had been inoculated with the culture from Mandan. The flecks developed into aborted uredinia which formed spores but never broke through the epidermis of the host. The uredinia remained in such a condition for two weeks, when the epidermis was ruptured with a needle and the spores transferred to other leaves of the same plant. Following the second inoculation, two very small uredinia appeared, but the leaves shriveled before the uredinia were fully formed, and the rust was lost. All other trials with the Mandan strain gave negative results. In one other experiment, infection resulted when Argentine was inoculated with urediniospores of the Fargo strain. A small uredinium appeared on one plant in twelve. In this case the uredinium was fully formed and the epidermis of the host was ruptured. A few days later several uredinia appeared on the same plant; and, two weeks after the first uredinium was noticed, the plant was heavily rusted. There were no signs of infection on any of the other plants even when inoculated with spores from the rusted Argentine plant. As only a single plant was rusted, it seemed probable that it may not have been Argentine. This explanation seems most plausible, because the experiment was

repeated many times, always with negative results. None of the other strains infected Argentine. This does not prove that there is no further specialization within *M. lini* on varieties of common flax, but it does indicate that, if there are physiologic forms, probably none of those occurring in this region are able to attack Argentine. The results are important when one considers the fact that Argentine is being used extensively as a resistant parent in flax breeding studies.

CONCLUSIONS

The facts obtained in these investigations indicate that epidemics of flax rust may occur each year under normal conditions. Cool, moist weather, the most favorable condition for the growth of flax, also is conducive to the development of flax rust. Spores germinate within a wide range of temperature. The spread of the disease is checked by hot, dry weather, as the maximum temperature for spore germination is 26–27° C. Uredinia are formed over a wide range of temperature, so that the supply of inoculum may increase rapidly, even at fairly low or high temperatures. Low temperature checks the development of the disease only slightly, since it merely delays the production of inoculum and does not inhibit spore germination. High temperature delays the formation of uredinia and inhibits spore germination so that the inoculum produced is not effective until a return of favorable conditions.

Spores must be in contact with water in order to germinate, but the fact that the pathogene can enter the host when abundant moisure is supplied for only three hours indicates that either light showers or dews may furnish sufficient moisture for infection. If plants are wet for only a short time, therefore, they may become infected.

Light seems to have little effect on spore germination and entrance of the germ tubes into the host. Infection can occur at any time of night or day. However, light is required for the formation of uredinia. Fair days favor the formation of uredinia, and a succession of cloudy days lengthens the incubation period somewhat; but apparently there is always enough light to enable the rust to develop well in nature.

The fact that *M. lini* is not restricted to common flax suggests the possibility that wild flaxes may be dangerous sources of inoculum in flax-growing areas. So far as determined, *L. lewisii* need not be feared, for the rust occurring on it probably will not attack the cultivated species. On the other hand, the wild species, *L. rigidum*, is attacked by the rust from common flax and may prove to be a source of infection for the cultivated crop.

The use of resistant varieties affords the only practical means of controlling flax rust. So far, Argentine has proved to be practically immune

from rust, apparently possessing a true physiologic resistance. Uredinial germ tubes of the fungus enter the resistant Argentine as well as the susceptible Winona flax. Argentine is hypersensitive to the pathogene, which kills some of the host cells quickly and is then unable to develop further. The evidence obtained by the writer indicates that in the principal flax-growing states there are no physiologic forms which can attack Argentine.

SUMMARY

1. A study was made of the factors affecting infection and development of *Melampsora lini* (Pers.) Lev.

2. The cardinal temperatures for the germination of aeciospores and urediniospores are 0.5° C., 18° C., and 26–27° C..

3. Aeciospores and urediniospores germinate readily in distilled water. The presence of host tissue does not stimulate germination appreciably.

4. Aeciospores begin to germinate at the end of 45 minutes at optimum temperature, each spore sending out from one to six germ tubes.

5. Urediniospores begin to germinate after 1½ hours, each spore sending out one or more branched, reddish-orange germ tubes, one of which develops more rapidly than the rest.

6. Urediniospores germinate equally well in light and in darkness.

7. Under favorable conditions, urediniospores retain their viability almost three months.

8. Urediniospores lose their viability more rapidly at high temperatures than at low ones.

9. Urediniospores retain their viability longer at relative humidities of 40 per cent and 60 per cent than at relative humidities of 20 per cent and 80 per cent.

10. Teliospores require a rest period before germination. Attempts to shorten the rest period failed.

11. Uredinial germ tubes enter the host through the stomata.

12. The uredinial germ tubes enter resistant hosts as well as susceptible ones, but the pathogene kills some of the cells of resistant hosts and then cannot develop further.

13. Uredinial infection occurs if inoculated plants are kept in moist chambers for 3 hours or more.

14. Light is necessary for the formation of uredinia. Increased light hastens formation of uredinia, while decreased light lengthens the incubation period.

15. Uredinia appear at temperatures ranging from 7 to 30° C. Severe infection occurs at 16–22° C. Slight infection occurs at 7–14° C., and at 26–30° C.

16. The intensity of infection is directly proportional to the luxuriance of growth of the host. Plants supplied with phosphates grew more luxuriantly and were more severely rusted than plants supplied with nitrates or sulphates.

17. The pathogene penetrates the cortical tissues of the flax and often attacks the fibers. The hyphae do not extend into the xylem.

18. Urediniospores of *Melampsora lini* from common flax do not infect the wild flax, *Linum lewisii*; and urediniospores from *L. lewisii* do not infect the common flax.

19. Urediniospores of *M. lini* from common flax infect *L. rigidum* heavily.

20. The variety Argentine was immune from all the strains of *M. lini* used in these studies.

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RELATION OF INTERNAL COB DISCOLORATION TO YIELD IN CORN

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The diseases of corn referred to by Holbert and Hoffer (1, 2) as the root, stalk, and ear rot diseases are very prevalent in Maryland. The estimated annual loss to the corn growers of the state from these diseases is about 10 per cent of the crop, which amounts to approximately 2,500,000 bushels. These diseases are prevalent in all parts of Maryland, but they cause the greatest losses in the poorer sandy and clayey soils.

Plans were made in 1921 to conduct demonstrations in the control of the root, stalk, and ear rot diseases, following the recommendations of Holbert and Hoffer (1, 2). It was found that only a few of the corn growers were willing to use the germinator to test all of their seed ears; on the other hand, many of them were already following most of the other recommendations of Holbert and Hoffer, with the exception of discarding ears with discolored butts.

FIG. 1. An ear of corn practically free from internal cob-discoloration (center), and two ears showing internal cob discoloration. The center of the ear at the left is also hollow. The discolorations may be red, pink, gray, or brown; they may involve only the vascular tissue, or may extend to the surrounding tissues.

When the first seed corn selections were made in this project, some of the ears which showed no butt discoloration were broken, and discoloration

was found in other parts of the cob. It was soon found that many cobs were discolored in the interior without showing any discoloration at the butt. The butts and tips were then chopped from a number of ears; some were found to be discolored internally at the butts and not at the tips, some at the tips and not at the butts, and others were discolored all the way through, from butt to tip.

Plans were made for conducting a series of tests to determine whether the red, pink, gray, brown, and other discolorations found in the vascular and surrounding tissues of the interior of the cob were in any way related to yield and to root, ear, and stalk rot diseases (Fig. 1); and, also, whether

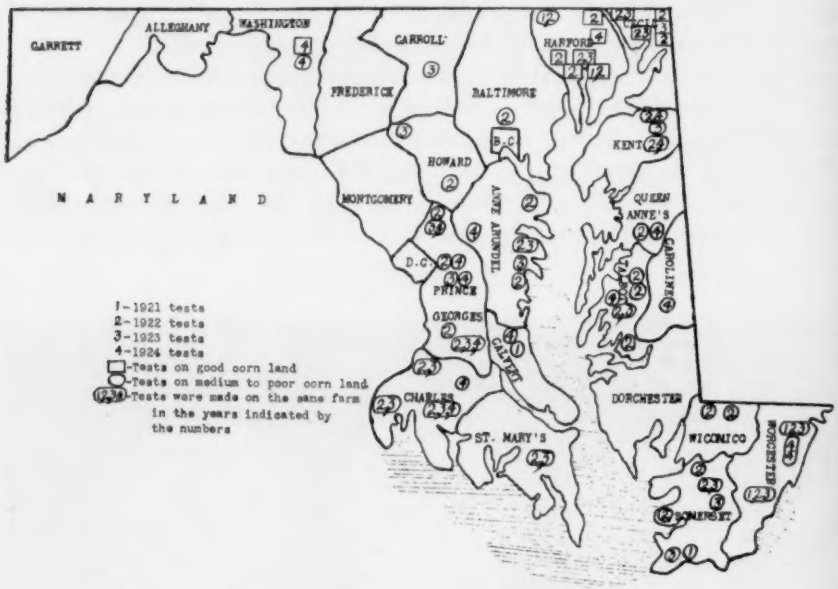

FIG. 2.—Location of corn root rot tests.

freedom from these internal cob-discolorations is an inherited character. These tests have been made in various parts of the State (Fig. 2) during the past four years.[1] They represent many different soil types and a wide range of climatic conditions. In some tests, pure-bred varieties, such as Reid's Yellow Dent, Improved Lehming, Collier's Excelsior, Johnson County White, and Boone County White were used; in other tests, mixed white and yellow dent varieties were used. In only one test, conducted on the Maryland Experiment Station grounds, were yield records obtained

[1] The writers were assisted in conducting these tests by other members of the Departments of Pathology and Agronomy and by the county agents.

with sweet corn. In addition to 90 tests in which accurate yield records were obtained, many other tests were made in which the yield records were estimated and field observations were recorded. In almost all of these additional tests, the results reported by the county agents and the growers were similar to those of the tests in which accurate records were obtained.

TABLE 1.—*Relation of internal cob-discoloration to yield in corn*

Year	No. of tests	No. of Counties	Av. yield of corn from cob without discoloration	Av. yield of corn from discolored cob	Average increase per cent	Average increase per cent	No negative results
1921 total	6	4	51.49	39.57	11.92	30.10	0
1922 total	39	15	67.19	58.37	8.82	15.11	4
1923 total	23	12	62.39	55.23	7.16	12.97	1
1924 total	25	11	55.18	46.45	8.73	18.79	2
1921 V.G.*	1	1	63.3	60.4	2.9	4.8	0
1921 M.P.**	5	4	49.03	35.41	13.62	38.46	0
1922 V.G.	9	3	88.91	82.66	5.63	6.81	2
1922 M.P.	30	15	60.46	51.08	9.38	18.36	2
1923 V.G.	3	2	81.42	78.25	2.57	3.28	0
1923 M.P.	20	11	59.49	51.78	7.71	14.90	1
1924 V.G.	2	2	63.45	61.52	1.93	3.13	1
1924 M.P.	23	10	54.46	45.14	9.32	20.64	1

(V.G.*) Very good corn land.
(M.P.**) Medium to poor land.

PROCEDURE

In each test, seed corn which had been selected by the grower was carefully culled. Off-type ears were discarded, as well as ears with moldy cobs or kernels; shredded shanks; blistered, checked, dull, soft, or starchy kernels; and discolored germs.

Instead of shelling the butts and tips, which is the usual practice, they were cut off. After the removal of the butts and tips, further examination of the grains was made for undesirable qualities. The selected ears were then divided into three or more lots, according to the intensity of internal cob-discoloration. The ears showing little or none were put into lot 1; those showing slight discoloration were put into lot 2; and those showing intense discoloration were put into lot 3. The amount of internal cob-

discoloration was found to vary greatly in different strains of corn, but the average amount which could be placed in lot 1 was approximately 10 per cent. A few strains were found in which 80 per cent of the ears could be placed in lot 1; whereas other strains were found in which practically all of the ears had medium to intense internal cob-discoloration.

In every test, a plot of lot 1 and another of lot 3 were planted. In some tests a plot of lot 2 also was included. A special effort was made to plant the corn where soil, drainage, and other conditions were uniform. Yield records were computed by weighing the corn from equal areas (about one-fourth acre) in each plot.

RELATION OF INTERNAL COB-DISCOLORATION TO YIELD

Yield records were obtained in 89 tests with field corn, and in one test with sweet corn. In 86 of these tests the corn planted from ears with practically no internal cob-discoloration gave a larger yield than the corn planted from ears with intense internal cob-discoloration. The results given in tables 1 and 2 show a decided correlation between internal cob-discoloration and yield, and this is more pronounced on sandy and clayey soils than on the best corn land.

TABLE 2.—*The relation of internal cob-discoloration to yield in sweet corn*

Internal cob-discoloration	Yield per acre in pounds	Total weight of dry ears in pounds	Increase over ears with most internal cob-discoloration	
			Pounds per acre	Per cent
Practically none	95.4	1431	176	14
Medium	90.8	1362	107	8.5
Intense	63.7	1255		

In some of the tests, three or four plots were planted, according to the intensity of the internal cob-discoloration. In almost every case there was an apparent correlation between the intensity of internal cob-discoloration and the yield. Some of the more striking instances are indicated in table 3.

INTERNAL COB-DISCOLORATION REDUCED BY SELECTION

In a large number of the tests, the selection for freedom from internal cob-discoloration was continued with the same corn grower for a period of from three to four years. In every instance the percentage of ears with internal cob-discoloration, as well as intensity of internal cob-discoloration,

was reduced by selection. Each year the seed ears were selected from the plots grown from ears with the least internal cob-discoloration. These seed ears were then sorted according to the intensity of cob-discoloration. Some of the most striking results are summarized in table 4.

INTERNAL COB-DISCOLORATION

The cause and nature of internal cob-discoloration have not been determined by the writers, but a number of investigators have made observations and studies which have a bearing on the subject. Holbert and Hoffer (2) state that "most ears have shank attachments pink, slightly pink or brown colored, or cracked or shredded, have grown on stalks more or less diseased," and that "such ears usually are diseased." In reference to the corn root rot diseases, Manns and Adams (3) state that "other symptoms

TABLE 3.—*The relation of intensity of internal cob-discoloration to yield in field corn*

Year	County	Plot and cob-discoloration	Bushels per acre	
			Yield	Yield from No. 1
1921	Harford	1	68.0	
		2 +[a]	50.8	17.2
		3 ++	42.6	25.4
1922	Anne Arundel	1	60.1	
		2 +	50.0	10.1
		3 ++	41.66	18.44
1922	Talbot	1	73.4	
		2 +	61.61	11.79
		3 ++	53.91	19.49
		4 +++	52.56	20.84
1923	Kent	1	54.48	
		2 +	50.30	4.18
		3 ++	34.74	19.74
1924	Anne Arundel	1	38.2	
		2 +	23.0	15.2
		3 ++	23.0	15.2

[a] + = relative amount of internal cob-discoloration.

which may indicate infection are discolorations of the cob at the butt or in the center. Such discolorations may be pink, brown, black, or yellowish." Kiesselbach (4) made germination tests from ears with yellow, pink, brown, and normal shanks in order to determine the percentage of kernels which were diseased and rotting after testing. He concluded that brown shank

discoloration was somewhat indicative of the corn root rot disease, and that pinkish and yellowish shank discolorations were not. Hoffer and Carr (5) found that the brown to brownish-purple discolorations which occur in the vascular plate tissue in the nodes of the stalk and shank and the scutellum of the kernels could be produced artificially by injecting solutions of certain iron and aluminum salts into the plants, and that "these artificially induced

TABLE 4.—*The effect of seed selection on the amount of internal cob-discoloration in corn*

Year	County	Per cent of seed ears with little or no internal cob-discoloration	Per cent of seed ears with internal cob-discoloration
1922	Cecil	75	25
1923		80	20
1924		67	33
1922	Charles	98	2
1923		90	10
1924		45.2	54.8
1922	Charles	95	5
1923		93.7	6.3
1924		56.8	44.2
1921	Carroll	85	15
1922		80	20
1923		70	30
1924		50	50
1922	St. Mary's	93	7
1923		70	30
1924		58	42
1921	Worcester	95	5
1922		80	20
1923		75	25
1924		3	97

changes in the plant parts closely resemble the phenomena which develop in the plants growing in fields under conditions favorable to the root rots." They also concluded that "corn plants showed marked differences in the quantities of aluminum and iron salts which are absorbed by them" and "these differences develop when the salts of iron and aluminum are available in subtoxic concentrations in the soil." These differences "are believed to be due to specific selective capacities of different plants to absorb

available aluminum and iron salts from the soil." They state that "resistance to the root rots in corn plants can be defined, first, by the respective abilities (inherited) of the plant to absorb materials present in the soil solution, and, second, by the relative concentrations of deleterious ions presented to the roots in soil solution."

Holbert, Burlison, Koehler, Woodworth, and Duncan (6) state that "there is much variation in color and in intensity of color of cob interiors either when viewed in cross-section after the cobs have been broken or when viewed in longitudinal section after they have been sawed longitudinally. Interior cob-coloring variations prevail in most open-pollinated strains of corn, even in those that have been selected rather closely for several years. It seems probable in many cases that red, pink, and brown colorings in the shank are due simply to lodgment in the shank attachment of the same pigment found in the vascular cylinder of the cob, and do not indicate infection. Hence, in observing the nature of the shank attachment for the selection of seed, it is very important to distinguish between normal colorations and discolorations resulting from decayed tissues. In describing a plate[2] in which internal cob-discoloration is illustrated, they state that "in the coloring of the interior of the cob, considerable natural variation occurs. In some cases the coloration extends to the point of attachment between the cob and shank, and thus gives the shank attachment a pink or brown appearance. In such cases the pink or brown shanks are normal." Thus they seem to be of the opinion that the internal cob-discolorations are normal when they do not result from infection and when they extend to the point of attachment between the cob and shank. Since the vascular system in the cob is a continuation of the vascular system in the shank, the internal discolorations which extend to the point of attachment between the cob and shank would seem to be caused by the same iron and aluminum compounds as those described by Hoffer and Carr (5). According to Holbert et al (6), the internal discolorations which do not extend to the point of attachment between the cob and shank result from infection by various organisms. If these conclusions are correct, internal discolorations resulting from both causes are being discarded by the Maryland method of chopping off the butts and tips and examining the interior of the cob at both ends.

In some of our tests, the symptoms attributed by several investigators to the root, ear, and stalk rot diseases, such as small and less vigorous plants, fallen and broken stalks, uneven stand, premature death, broken shanks, etc., were less pronounced in the plot grown from ears with least internal cob-discoloration than in the plots grown from ears with most internal cob-

[2] Plate V, page 378.

discoloration. This is very well illustrated by one test made in Harford County, where 248 down stalks were found in 400 hills in the plot grown from seed produced on ears with the most internal cob-discoloration; whereas only 64 down stalks in 400 hills were found in the plot grown from seed produced on ears with the least internal cob-discoloration. Similar conditions were observed in many other tests.

On a farm in Cecil County, a strain of yellow dent corn was found in which approximately 75 per cent of the ears were practically free from internal cob-discoloration. It has other desirable qualities, among which are a medium-sized cob and grain of a deep yellow color. This strain of corn has been planted in various parts of the state. In most places it has produced a uniform stand relatively free from fallen and broken stalks. In almost every case where it has been tested, the yield from this strain has been greater than the yield from the local variety planted in the same field.

SUMMARY

The results of 90 tests conducted over a period of four years seem to indicate the following:

1. The yield from seed corn on cobs free from internal discoloration is greater than the yield from seed corn on cobs with internal discoloration, provided other conditions are similar.

2. This difference in yield is greater on poorer corn land than it is on good corn land.

3. The differences in yield vary with the intensity of internal cob-discoloration; the slighter the internal discoloration, the greater the yield.

4. Internal cob-discoloration can be decreased by selection. It has been eliminated almost entirely in four years, by planting each year seed of ears free from internal cob-discoloration.

5. Corn grown from ears with the least internal cob-discoloration seems to have a more uniform stand, a smaller number of fallen and broken stalks, and fewer prematurely killed plants than corn grown from ears with the greatest amount of internal cob-discoloration.

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BLOSSOM BLIGHT OF THE PEACH

JOHN W. ROBERTS AND JOHN C. DUNEGAN

The brown rot disease of the peach caused by *Sclerotinia cinerea* (Bon.) Schröt, is, as its name suggests, best known as a rot which occurs on the ripe or nearly ripe fruit. There are, however, other manifestations of the disease, of which the most prominent is the form known as blossom blight, readily recognized by a withering of the blossoms and the production of conidial tufts on the diseased floral parts.

In central Georgia, blossom blight frequently causes severe damage, not so much by reducing the set of fruit as by establishing numerous sources of infection in the orchard, which later make it extremely difficult to prevent the fruit from rotting. Since the blighted blossoms frequently adhere to the twigs until after the fruit ripens, producing immense numbers of conidia during periods of rainy weather, they become a direct menace to the maturing fruit.

In other regions, such as parts of Delaware and the eastern shore of Maryland, the blighted blossoms are often so numerous as to curtail appreciably the size of the crop; but, under Georgia conditions, direct loss from this phase of the trouble is of infrequent occurrence.

That *S. cinerea* can produce blossom blight on peach trees has been known for many years. Smith (10, 11), in 1889, was among the first to mention the destruction of peach blossoms by this fungus. Since that time, Quaintance (8), Scott and Ayres (9), Jehle (4), McCubbin (6), Cook (2), McClintock (5), and many others have discussed blossom-blight of the peach, both as a destroyer of blossoms and in relation to the subsequent infection of the twigs and fruit.

Previous to Norton's (7) demonstration of the existence of the ascogenous stage of the fungus it was generally believed that infection of blossoms was due to conidia, although Smith (11) had pointed out that it was hard to understand how there could be a severe outbreak of blossom blight after a year in which there had been no brown rot of the fruit and no twig blight to transmit the disease. Since the publication of Norton's investigations, it has been generally assumed that ascospores are the principal source of infection of the blossoms, because they are usually produced in large numbers during the flowering period of the peach. Cook (2), however, reports severe cases of blossom-blight in orchards practically free from mummied fruit. He considers the infections to be related to the presence of cankers on the twigs. Scott and Ayres (9) state: "These ascospores, as well as

the conidia, serve to infect the blossoms." McClintock (5) produced characteristic blight by spraying peach blossoms with water containing suspended conidia obtained from mummied fruits. The inoculated blossoms were then covered with paper bags. McClintock worked with trees growing out of doors, and apparently no controls were used.

Experiments also have been made to determine the stage at which peach blossoms are most subject to infection and the most susceptible parts of the flower. Jehle (4) states: "Infection seemed to begin at the calyx or shuck and spread from there into the stamens and pistil, accompanied by a withering and browning of the diseased portion. Sometimes only the shuck becomes diseased and this is then shed without further injury to the pistil, but usually the entire flower becomes infected . . ." McCubbin (6) observed infected calyces of peach blossoms. Barss (1), in a discussion of blossom blight of stone fruits, states: "Petals or calyx lobes and likewise the stigmas of the flower are very subject to infection. The first sign is a small brown spot on the petal, calyx lobe, or tip of the pistil. This discoloration spreads rapidly if the weather continues moist. The disease eventually kills the parts attacked and begins to invade the base of the flower, which then turns dark."

The experiments reported upon in this paper were made chiefly for the purpose of determining the susceptibility of blossoms to infection by conidia and ascospores and the relative susceptibility of the different parts of the flower. The experiments by the senior author were made at Washington, D. C., and those by the junior author at Fort Valley, Georgia.

EXPERIMENTS AT WASHINGTON, D. C.

For the experiments reported upon here, the apothecia of *S. cinerea* and the peach branches bearing blossoms were obtained from Arlington Farm, Virginia, in the spring of 1924. In all cases the conidia were from cultures grown on four per cent potato agar, the same physiologic form being used throughout. This particular physiologic form, obtained from a single ascospore produced in an apothecium from a mummified plum, grows very rapidly and produces conidia more readily than any other grown by the senior author. The ascospores and conidia respectively were applied to the blossoms in watery suspension by means of an atomizer, except that, in the experiments of April 16, additional ascospores were applied by exposing the blossoms to a cloud of them expelled directly from apothecia. The control branches were sprayed with distilled water only. In all experiments there were three lots, receiving different treatments and kept separate under individual bell jars:

Lot 1. Sprayed with ascospores suspended in distilled water.
Lot 2. Sprayed with conidia suspended in distilled water.
Lot 3. Control. Sprayed with distilled water.

In each experiment the branches were cut in the orchard and brought into the laboratory before the blossoms had opened. Inoculations were made in the laboratory when nearly all the blossoms had opened. It was believed that the chances of accidental infection would be much less than when the branches with opened blossoms were taken directly from the orchard. At any rate, as shown by the results, there was little or no accidental infection. The number of blossoms in each lot varied from 39 to 215.

For the first and second experiments begun on April 12, branches of peach seedlings were used. For the third and fourth experiments, begun on April 15 and 16 respectively, branches of the Carman variety, the blossoms of which are moderately susceptible to the blight, were used. The results of these experiments are shown in table 1.

TABLE 1.—*The results of inoculating blossoms of peach seedlings and blossoms of the variety Carman with Sclerotinia cinerea at Washington, D. C., in 1924*

Lot	Inoculum	Percentage of blossoms blighted after 4–5 days			
		Experiment 1 (seedling)	Experiment 2 (seedling)	Experiment 3 (Carman)	Experiment 4 (Carman)
1	Ascospores suspended in distilled water	10	0	93	88
2	Conidia suspended in distilled water	40	20	90	56
3	Control, distilled water	0	0	0	0

All the blighted blossoms bore an abundance of the conidia of *S. cinerea*. All the blossoms of lot 3 (the controls) in all the experiments remained normal to the end except for the falling of a few petals. In lots 1 and 2, only those which were well opened were attacked by the fungus. In experiments 1 and 2, the infections were mainly on the stamens and pistils, the anthers and stigmas being the first to show the blight and the first to show production of conidia. These parts of the young blossoms, being rather closely pressed together, retained for some time the water in which the conidia or ascospores were suspended. Opened anthers seemed very susceptible. In subsequent experiments, all parts appeared about equally susceptible, the sepals and petals as well as the stamens and pistils being

literally covered with conidia in the few days allotted to the experiments. The differences in the results of the earlier and the later experiments may have been purely accidental, but in the later experiments the blossoms were more widely opened and received the suspensions of ascospores and conidia more directly upon their inner surfaces.

On drying, blighted blossoms with uninfected peduncles fell off, but those with infected peduncles adhered tightly to the twigs as under natural conditions in the orchard.

The inoculation experiments show that at a moderate temperature (25° C.), and under conditions of extreme moisture, only a few days are required for the development of blossom blight. In 48 hours small brown spots began to appear on the various parts, and the formation of conidia followed very closely. Blossoms only partly opened were not easily infected, and those escaping infection after being inoculated were usually in this condition. The presence of conidia on the blighted parts made reisolation of the fungus seem a useless procedure, but it was performed for each lot inoculated.

EXPERIMENTS AT FORT VALLEY, GEORGIA

Ascospores of *S. cinerea* from fresh apothecia and peach branches bearing blossoms were obtained from orchards in the immediate vicinity of Fort Valley.

Conidia were obtained by flooding with sterile water young cultures of the fungus isolated from a peach and growing on potato dextrose agar. The conidia were collected by centrifuging the water suspension and were applied directly to the stigmas with a platinum loop, or the water suspension was sprayed on the blossoms with an atomizer.

Ascospores were secured by crushing the mature apothecia in sterile water. The ascospores were obtained from the crushed material in water by centrifuging and were applied directly to the stigmas with a platinum loop, or the water suspension was sprayed on the blossoms with an atomizer.

Sterile water was either applied with a platinum loop directly to the stigmas of the blossoms used as checks or was sprayed onto all parts.

The twigs bearing the inoculated blossoms and the checks were placed in individual moist chambers to provide conditions favorable to the development of the fungus.

The experiments of 1924 were conducted with the varieties Hiley and Uneeda. The blossoms were inoculated on the stigmas only. Typical blossom blight developed on both of these varieties following inoculation of the stigmas with either conidia or ascospores. The first signs appeared on the stigma, but infection progressed rapidly down the style and finally involved the whole blossom. The anthers and sepals seemed to be slightly more susceptible than the petals, but abundant spore tufts were finally

produced on all the floral parts. Occasionally the fungus continued downward through the peduncle and formed small cankers on the twigs. When this occurred, the blossoms adhered to the twigs as under natural conditions in the orchard.

The check blossoms remained normal in practically all cases, save for the dropping of the petals. The few that developed blight were believed to have received chance infections prior to their removal from the orchard.

Symptoms typical of blossom blight as found in the orchard were produced repeatedly as the result of stigma inoculations with conidia and ascospores. However, since the two kinds of spores may settle on any part of the blossom under natural conditions in the orchard, additional inoculations were made in 1925 by spraying all parts of the blossoms with a water suspension of the spores.

TABLE 2.—*The results of inoculating blossoms of the peach varieties, Carman and Uneeda, with Sclerotinia cinerea at Fort Valley, Georgia, in 1925*

Lot	Inoculum	Percentage of blossoms blighted after 5–6 days	
		Experiment 1 (Carman)	Experiment 2 (Uneeda)
1	Ascospores suspended in distilled water.	61	—
2	Conidia suspended in distilled water.	93	93
3	Control, distilled water.	0	0

The first experiment was started on March 9, and the second on March 14, 1925. For the first experiment, twigs of the variety Carman bearing open blossoms were selected from an orchard in which no apothecia could be found. For the second experiment, blossoms of Uneeda, a variety quite susceptible to the disease as it occurs in the orchard, were used. Twigs bearing open blossoms and numerous buds had been brought to the laboratory on the 10th and all the open blossoms removed. By March 14 a number of the buds had opened in the laboratory. Only conidia were used in this experiment, as apothecia with viable ascospores were no longer available.

For the first experiment, the twigs were divided into three lots and treated as follows:

Lot 1. Sprayed with a suspension of ascospores in distilled water.
Lot 2. Sprayed with a suspension of conidia in distilled water.
Lot 3. Control. Sprayed with distilled water.

In the second experiment lot 1 was of course omitted. The number of blossoms in each lot varied from 88 to 129.

The twigs standing in jars of water were placed in individual iceless refrigerators as devised by Hunt (3). The results of these experiments are shown in table 2.

The stamens, anthers and sepals were in most cases covered with conidial tufts. The petals and stigmas, while they were quite susceptible, did not seem to serve as a locus for conidial production as readily as the other parts. The anthers were particularly susceptible.

CONCLUSIONS

The inoculation experiments reported here indicate that both ascospores and conidia can infect any part of the peach flower, each possessing about the same degree of pathogenicity. As either or both may be present when peaches are in blossom, they are both to be regarded as dangerous, and pathologists should consider the prevalence of both in relation to the disease.

SUMMARY

Inoculation experiments carried on at Washington, D. C., and Fort Valley, Georgia, show that the conidia and the ascospores of *Sclerotinia cinerea* (Bon.) Schröt. are capable of causing a blossom blight of the peach.

All parts of the open blossoms may be attacked. Direct infections of the stigmas, anthers, petals, and inner surfaces of the sepals were observed.

It is concluded that either ascospores or conidia, or both, may be responsible for outbreaks of blossom blight in peach orchards.

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THE DISTRIBUTION OF CRANBERRY FALSE BLOSSOM

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The economic importance of "false blossom" of the cranberry (*Vaccinium macrocarpum*) has made necessary a careful field study of the disease for the purpose of discovering possible resistant varieties, or conditions of culture under which the severity of the disease had been reduced. This survey has yielded some interesting additions to our knowledge of the present distribution and manner of spread of the disease. This paper summarizes the available knowledge of the distribution of false blossom and contains information obtained since the last publication on the subject, to which reference may be made for citations of the literature on this disease.[1]

The cause of false blossom has not been determined. Most of those who are now actively studying cranberry disease problems in the field, however, have come to agree that it behaves like an infectious disease; and the present writers are much more concerned with its control than with its cause.

THE DISTRIBUTION OF FALSE BLOSSOM IN NEW ENGLAND

In the Cape Cod region of Massachusetts, false blossom is now known to occur on 52 bogs. The diseased bogs are largely in Plymouth County. On at least six of them the disease first was found on vines brought from Wisconsin. A surprising number of the remaining bogs which now show false blossom are known to have been planted, at least in part, with vines from bogs on which the disease had already become established. Many of these originally diseased bogs contained at one time either Wisconsin vines or vines of the Massachusetts variety, variously known as Holliston, Bacheldor, Mammoth, and Small's Number One. The last-named variety seems to be very susceptible to false blossom and is generally diseased wherever found in Plymouth County. No false blossom could be found, however, in the original plantings of this variety in the town of Holliston, which led Dr. Franklin[2] to suggest that at least some of the vines which are called Hollistons on Cape Cod might really be Bennetts, a Wisconsin variety. Support is given to this theory by the fact that the two varieties are so similar in appearance that we are not yet able to distinguish them, and by the fact that Emulous Small, who was one of the most active Cape Cod

[1] Stevens, Neil E. Field observations on false blossom of the cultivated cranberry. Phytopath. **15**: 85–91. 1925.

[2] Franklin, H. J. Report of the cranberry station for 1919 and 1920. Mass. Agr. Exp. Sta., Bul. 206: 149–168. 1921.

growers in introducing and distributing Holliston vines, was a life member of the Wisconsin Cranberry Growers' Association and an intimate friend of at least one Wisconsin grower. To present in detail the information which has been accumulated on the spread of this disease from bog to bog in Massachusetts would occupy too much space. One or two examples may, however, be given.

Outside Plymouth County, a severe infection of false blossom on the Howes variety was found recently by Dr. Franklin on a bog near New Bedford, in Bristol County. The vines for planting this bog came from a diseased bog near East Wareham, Mass. In Chatham, Barnstable County, Dr. Franklin also found scattered vines affected with false blossom on one bog planted with a local variety, Smiths, and with Howes; but a part of the Howes proved to have come from a small bog which in turn had been planted from a diseased bog in Carver. Careful search on other bogs in the neighborhood, containing the Smith variety, as well as other varieties, failed to show any false blossom. As yet false blossom has not been found on any of the small bogs of the "lower cape" (Barnstable County) which are planted wholly to local varieties.

Genuine "wild bogs" are now rare in the Cape Cod region, but they are fairly common in Maine and New Hampshire. Careful search on many of these wild bogs, whenever opportunity offered during the past five or six years, has failed to show any false blossom on any wild bog in this region. This is in striking contrast to the distribution of the fungous diseases of the cranberry. Many of these diseases are very widely distributed on wild bogs. Such fruit-rot fungi as *Fusicoccum putrefaciens* Shear, for example, have been found in cranberries gathered on wild bogs in northern Maine, and in mountain cranberries (*V. vitis-ideae*) from several remote localities. Rose bloom (*Exobasidium oxycocci* Rost) is also widely distributed on cranberries throughout this region and was found several years ago by Dr. Shear on a tiny patch of *V. oxycoccus* near Lake of the Clouds, only a little over a mile from the summit of Mount Washington in New Hampshire. False blossom has not yet been found on cranberries in either New Brunswick or Nova Scotia, Canada, although there are cultivated bogs in the latter province. The absence of false blossom on wild bogs in New England and the adjacent parts of Canada, together with the fact that in many cases the infection is known to have come from bogs already diseased, suggests that false blossom is not native in the region but was introduced from Wisconsin.

DISTRIBUTION OF FALSE BLOSSOM IN NEW JERSEY

Our knowledge of the occurrence of false blossom is less complete in New Jersey than in New England. Through the generous assistance of Mr. C.

S. Beckwith, of the New Jersey Experiment Station, one of the writers has been able to make a fairly satisfactory study of bogs in different regions of this State, and the information thus obtained seems to justify the following statement concerning conditions in New Jersey. False blossom is widely distributed in New Jersey, though only in a very few cases has it become markedly destructive. It was found on almost every bog containing Howes, the most commonly planted variety which comes from outside the state. Unlike Cape Cod, New Jersey has large areas of native, more or less mixed vines, some of which are cultivated and some wild. False blossom is very rare on such bogs. In fact, although "Native Jerseys" have proved very susceptible to false blossom on certain bogs where they were grown close to infected Howes or Hollistons, false blossom has not yet been found on any bog containing only Native Jerseys.

On at least three bogs on which false blossom now exists, vines from diseased bogs are known to have been planted. In one case the diseased vines had come from Wisconsin, and in two cases from Massachusetts. The fact that false blossom is widely distributed in New Jersey on vines of Massachusetts or Wisconsin varieties, but is unknown on "Jersey" vines except where these are planted near introduced varieties, seems best accounted for by the supposition that the disease is not native in New Jersey but was introduced both from Massachusetts and Wisconsin.

THE FALSE BLOSSOM SITUATION IN WISCONSIN

In the course of a survey of false blossom distribution in Wisconsin during the summer of 1925, the disease was found on 48 of the 61 cultivated bogs[3] examined. Of these, at least 20 have infections severe enough to materially affect the crop. It probably occurs on other bogs where it was not found.

False blossom is believed by some of the older growers to have been present in the State at least as early as 1885. It did not, however, receive serious attention as a disease until many years later. About 1900 a considerable planting was made in the Mather district of three varieties of vines from the Berlin region, namely, Palmeter, Bell and Bugle, and Metallic Bell. By 1906 false blossom had become prevalent in these varieties, and since then it has become so severe that their cultivation has been materially reduced. Contrary to the general belief that false blossom is on the wane in Wisconsin, a careful comparison of the same varieties on the same bogs over a period of five years seems to show an increase in the amount of disease in 1925 over previous years. The apparent decline of the disease may well be due, not

[3] Cultivated areas of cranberries, which in other cranberry sections are known as *bogs*, are in Wisconsin generally referred to as *marshes*. We are here using the shorter word for the sake of uniformity.

to an actual decrease of false blossom on individual bogs, but to the fact that the most susceptible varieties are being replaced by those which are more resistant.

The severity of false blossom in the Mather district on varieties brought from near Berlin, Wisconsin, has given rise to the general belief that it was introduced into the Mather region on the Berlin vines. It is perhaps even more probable that the disease was already present in or near Mather and that the Berlin vines proved particularly susceptible. The observations in 1925 show at least that false blossom is less common in the Berlin region than in any other cranberry district of Wisconsin. It is probably significant that false blossom is more widely distributed in Wisconsin than in any other cranberry-growing state. Even more significant is the occurrence in Wisconsin of this disease on wild cranberry vines remote from cultivated bogs.

FALSE BLOSSOM ON WILD BOGS IN WISCONSIN

Wild cranberry vines are now very few in the Mather and Cranmoor regions apparently owing to the fact that drainage operations which have dried out the bogs have been followed by fires which destroyed the vines. In the northern part of the State, however, wild cranberry bogs may still be found. The time available for field work permitted the examination of but few of these wild bogs. Nevertheless, on two of them northwest of Spooner, false blossom was found in abundance. One is three miles and the other eight miles from a cultivated bog, and both are over two hundred miles from the region where false blossom is abundant on cultivated bogs. Indeed the disease was introduced into the nearest cultivated bog by diseased plants taken from one of these wild bogs. It seems impossible, then, for the disease to have been introduced into these wild bogs from cultivated ones. The only reasonable assumption is that it is indigenous.

CONDITIONS ON THE PACIFIC COAST

In Oregon, where the cranberry plantings were made largely with Wisconsin vines, false blossom is present on practically all the bogs. It is not serious, however, in this region. Mr. H. F. Bain, who has been studying cranberry diseases in Oregon and Washington for the last four years, is of the opinion that false blossom does not spread, or at least spreads very slowly, in that region; while Dr. Shear, who examined most of the Oregon bogs for false blossom in 1919 and again in 1924, reports a decrease during that period in the amount of the disease on the bogs observed. In Washington, false blossom is known to occur on only one bog. This was planted with vines from Wisconsin. The absence of false blossom on other bogs in Washington may be due in part to the fact that the disease seems to spread

very slowly in the Columbia River region, and also to the fact that a large part of the vines are McFarlins, a variety resistant to false blossom.

VARIETAL RESISTANCE TO FALSE BLOSSOM

As previously reported, there is no generally grown variety of cranberries which is free from false blossom. There is, however, a difference in the severity of the disease on different varieties. In Wisconsin, Palmeter, Bell and Bugle, Metallic Bell, Bennet Jumbo, Prolific (a Michigan variety), and the Mammoth (a Massachusetts variety, there called Holliston) have proved generally susceptible. The Searls is usually free from false blossom in the Cranmoor district. In the Mather region, however, and near Minong, Wisconsin, there are some very severe infections on this variety. The Searls has proved very susceptible to false blossom also when planted in Massachusetts. The McFarlin, however, while occasionally attacked by false blossom, has proved the most highly resistant of any variety, both in Massachusetts and Wisconsin.

CONTROL

At present, the most promising control method seems to be the regular spring and fall flooding of the bogs combined with general good culture. With this should go the elimination of the most highly susceptible varieties, and the planting of McFarlins in badly diseased areas on bogs where experience has demonstrated that this variety can be grown to advantage.

A promising means of control which is now being tried in Wisconsin, and the progress of which will be followed with interest, is the practice of holding the winter flowage very late (about July 1) for a year or two.

SUMMARY

"False blossom" of the cranberry has been found in Wisconsin on wild vines remote from cultivated bogs. It has not yet been found on wild vines in any other cranberry-growing region. The disease is also more generally distributed on cultivated cranberries in Wisconsin than in any other State.

The distribution of false blossom in New England and on the Pacific Coast seems to indicate that it was introduced into those regions by means of diseased vines brought from Wisconsin.

The disease apparently was introduced into New Jersey on diseased vines both from Massachusetts and Wisconsin.

The information now available suggests that false blossom first occurred in the United States on cranberries in Wisconsin and from there was spread to other cranberry-growing regions in shipments of diseased vines.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

RELATION OF STRAWBERRY FRUIT ROTS TO WEATHER CONDITIONS IN THE FIELD

DEAN H. ROSE

In an earlier paper[1] the writer presented the results of a two years' study of leather rot of strawberry fruits caused by a fungus apparently identical with *Phytophthora cactorum* Leb. et Cohn. The chief conclusion therein drawn, aside from that relative to the cause of the disease, was "that there is a close relation between leather rot and rainfall and that, in the region studied, the rot can be expected to reach a maximum within three to four days after heavy rains." The further statement was made that "temperature is important also, but its effect can not be determined without further study."

These conclusions were based on work done chiefly at Beebe, Arkansas, during the season of 1923. It is worth reporting, therefore, how they agree with those to be drawn from work done (also at Beebe, Ark.) during the picking season of 1924, especially since it has not been possible to continue the work in 1925.

Results obtained during 1923 and 1924, together with temperature and rainfall records, are summarized in tables 1 and 2, and shown graphically in figure 1. Weather notes are given in table 3.

The methods used during the second year's work were the same as those

TABLE 1.—*Percentages of leather rot, other rots, and total rot in strawberries used in holding tests, on first day after removal from storage, Beebe, Ark., 1924*

Kind of rot	Dates of picking; May, 1924														
	7	8	9	12	14	17	19	21	23	24	26	27	28	29	30
	Percentage of Rot														
Leather rot	----	----	----	----	----	----	----	----	----	----	3.0	0.7	0.5	1.9	0.3
Other rots	----	----	----	----	----	----	----	----	----	----	0.6	2.7	2.3	10.6	1.2
Total rot	0.7	0.9	0.0	0.8	0.4	0.2	0.4	0.0	0.1	1.8	3.6	3.4	2.8	12.5	1.5

[1] Rose, Dean H. Leather rot of strawberries. Jour. Agr. Res. 28: 357–375. 1924.

described in the earlier paper. Berries from the various experimental plots or from other fields around Beebe were held in the local cold storage plant for four days, then taken out, hand sorted, and counts made to determine the number of sound berries, the kinds of rot present, and the percentage of each. Since the percentages were not large at any time, and there were no observable differences among the various plots, the data from the experimental field are reported for the field as a whole and not for each plot separately. As in the earlier report, the percentage of rot is recorded (Table 1 and Fig. 1) for the day on which the picking was made, although it was not determined until four days later. Berries used from May 7 to 23 were of the Klondike variety; those used from May 24 to 30 were of the Aroma variety.

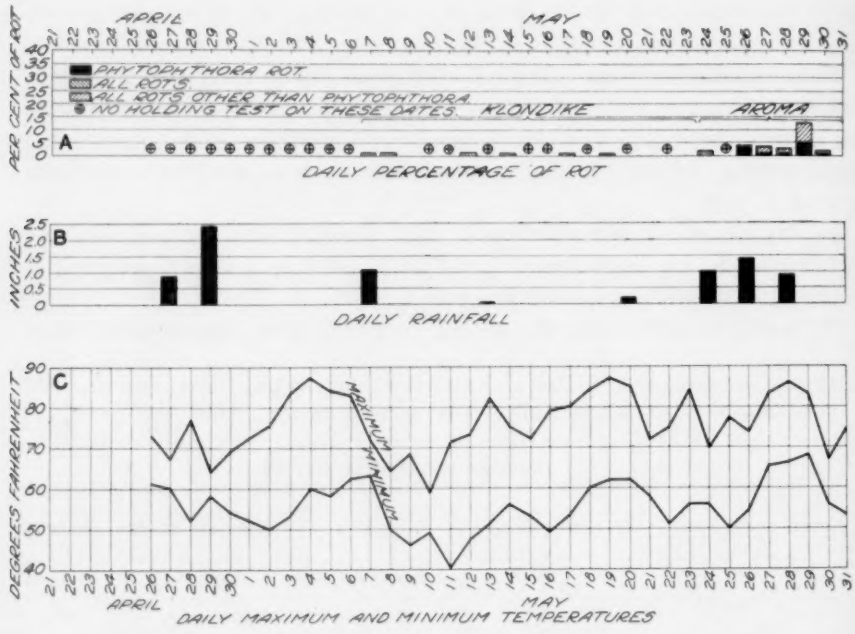

FIG. 1. A. Percentage of rot found, immediately after removal from storage, in various lots of strawberries held for four days at a temperature of 40–45° F. Percentages are entered for the day on which the berries were picked. B. Daily rainfall in inches. C. Daily maximum and minimum temperatures recorded at Beebe, Arkansas, by means of standard apparatus loaned by the United States Weather Bureau. Period covered, April 26 to May 31, 1924.

In 1924 there was almost no rot of any sort except during the last few days of May, when leather rot and other rots, chiefly Rhizoctonia rot, began

to appear in the field and in the packed fruit; even on these dates the percentages were very much lower than those reported for a large part of the 1923 season. It will be noted, however, that in 1923 the rainy periods during the picking season were followed by warm weather, while in 1924 all of the rains, with the exception of those on May 24 and 26, were followed by relatively cool weather. Therefore the inference seems justified that, in the region studied, rot of strawberries in the field (enlarging the earlier statement so as to cover leather rot and all other rots) is dependent upon two factors, rainfall and temperature. If rains are followed by cool weather, there is less danger of rot; if followed by warm weather, rot is likely to be serious. This conclusion is practically that arrived at on the basis of the earlier work, except that it includes a more definite and better documented statement on the relation of temperature to the incidence of rot. On one point the data for 1924 are less clear than those for 1923.

TABLE 2.—*Daily temperatures and rainfall, April 26 to May 31, 1924, at Beebe, Ark.*

Date	Temperature in degrees F.		Rainfall in inches	Date	Temperature in degrees F.		Rainfall in inches
	Maximum	Minimum			Maximum	Minimum	
1924							
April 26	73	61	0	May 14	75	56	0
27	67	60	.85	15	72	53	0
28	77	52	0	16	79	49	0
29	64	58	2.49	17	80	53	0
30	67	52	0	18	84	60	0
May 1	72	50	0	19	87	62	0
2	75	48	0	20	85	62	1.45
3	83	51	0	21	70	58	0
4	87	60	0	22	75	51	0
5	84	58	0	23	84	56	0
6	83	62	0	24	70	56	.9
7	72	63	.53	25	77	50	0
8	64	50	0	26	74	54	1.39
9	68	46	0	27	83	65	0
10	59	49	0	28	86	66	.88
11	71	40	0	29	83	68	0
12	73	47	0	30	67	56	0
13	82	51	0	31	74	53	0

There seems to be a lag in the appearance of rot following the rains late in May, but it is not so clearly defined as during the period May 14–18, 1923.

At no time in 1924 was leather rot as serious, compared with other rots, as it had been in 1923.

TABLE 3.—*Weather notes, Beebe, Ark., from April 26 to May 31, inclusive, 1924.*

April	26—Cloudy	May	14—Clear
	27—Cloudy and rain		15—Partly cloudy
	28—Cloudy		16—Partly cloudy
May	1—Clear		17—Hazy
	2—Clear		18—Clear
	3—Clear		19—Clear
	4—Clear		20—Cloudy
	5—Clear		21—Cloudy
	6—Cloudy		22—Clear
	7—Rain		23—Cloudy
	8—Cloudy		24—Partly cloudy
	9—Cloudy		25—Clear
	10—Cloudy		26—Cloudy
	11—Clear		27—Clear
	12—Partly cloudy		28—Cloudy
	13—Cloudy		29—Partly cloudy
			30—Clear
			31—Cloudy a. m.

OFFICE OF FRUIT DISEASE INVESTIGATIONS,
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PHYTOPATHOLOGICAL NOTES

International Problems in Plant Pathology: An Invitation.—The forthcoming International Congress of Plant Sciences (Fourth International Botanical Congress) is to be held at Cornell University, Ithaca, New York, U. S. A., from August 16 to 23, 1926. Invitations are being issued to all plant workers in the world to attend. The Congress will be divided into about twelve separate sections for the presentation of papers and the transaction of business of interest to the section. Plant Pathologists (the word is used in the restricted, or American sense) will organize in Section J. A formal program of invitation papers is being arranged. Ample time will be reserved for round-table discussions, field trips, exhibitions, etc.

Although it has been decided that no international legislation will be enacted at this congress, there will be opportunity in Section J (and doubtless in other sections as well) for the presentation of "proposals" for the consideration of the Section. Any person who wishes to make a proposal relative to any phase of plant pathology is invited to observe the following points:

The proposal should deal with a problem of international significance; it should be brief and concise; an oral or written argument should be prepared in advance; an abstract of not to exceed 500 words should be submitted to the Secretary not later than May 1, 1926. Proposals may be admitted to the program after May 1, only on condition that the author prepare an abstract in replica of about 100 copies in at least three languages.

The secretary for Section J is Donald Reddick, College of Agriculture, Ithaca, New York, U. S. A., to whom all communications regarding the business of the Section should be addressed.

A Simple Method of Inoculating Potatoes with the Spindle-tuber Disease.—A simple modification of the tuber graft method, by which an average of 90 per cent infection has regularly been obtained with spindle-tuber, has been found very advantageous. The same method also has been used with mosaic and leaf roll. A cork borer of 8–10 mm. in diameter is used to bore a hole through a healthy seed piece, and the core of tissue from an infected tuber obtained with a slightly larger cork borer is forced into the hole. Perfect contact of a relatively large surface is thus obtained, and inoculations can be made rapidly, three inoculated and one check plant usually being obtained from a healthy tuber. Double inoculations also can be made easily. The disease appears early, and the new tubers have distinct symptoms.—R. W. Goss, Nebraska Agricultural Experiment Station, Lincoln, Neb.

Verticillium Wilt of Tomatoes in California.—In the March, 1925, number of PHYTOPATHOLOGY, Mary K. Bryan called attention to a wilt disease of tomatoes which occurs in northern Ohio and Erie County, Pa., and which she has proved to be due to a *Verticillium*—probably *V. albo-atrum.* Hers is the only reference in the literature of America to a *Verticillium* wilt disease of tomatoes. She expressed the opinion that in regions where cool temperatures prevail this organism is responsible for much of the damage now attributed to *Fusarium vasinfectum,* since the symptoms of the wilts produced by the two organisms are identical.

In the San Francisco Bay region, California, a very serious wilt disease of tomatoes has been found to be due to a species of *Verticillium* resembling *V. albo-atrum.* In October, 1924, this organism alone was found associated with wilted plants in tomato fields in which as many as 90 per cent of the plants were destroyed. The fungus was isolated and its pathenogenicity proved by successful inoculations on tomatoes in the greenhouse.

The fungus has been found to be identical with the *Verticillium* which causes the so-called "black heart" of stone fruits, a widespread disease of considerable importance described in California by Czarnecki.[1]

It is also identical with the *Verticillium* which causes "blue stem" of bush fruits there, the Ranaree raspberry being particularly susceptible to attack.

Single spore cultures of the *Verticillium* isolated from typical cases of blue stem of raspberry, black heart of apricot and peach, and wilt of tomatoes have been used to inoculate tomatoes. The fungus from each of these sources attacked tomato plants with equal readiness, and typical wilt symptoms were produced. The fungus was eventually recovered by the usual methods from a large number of plants used in the experiments. Passage of the fungus from these same sources through Myrobalan plum seedlings and young Ranaree raspberry plants has been accomplished. Typical wilt symptoms manifested by these plants were produced, and the fungus was recovered in a number of instances.

Further discussion of the *Verticillium* wilt diseases of these economic plants in California is reserved for a later paper.—B. A. RUDOLPH, University of California Deciduous Fruit Station, San Jose, Cal.

Berberis fendleri, an Alternate Host of Puccinia graminis tritici.—*Berberis fendleri* Gray, the wild barberry, native to certain sections of the Rocky Mountains, for some time has been considered a host of two grass rusts. Arthur,[2] in North American Flora, reports this species of barberry not only

[1] Czarnecki, H. Studies on the so-called black heart disease of apricots. Phytopath. 13: 216–224. 1923.

[2] Arthur, J. C. N. Amer. Flora 7: 295–330. 1920.

as a host of *Puccinia montanensis,* but also lists it as an alternate host of *P. graminis.* However, no reported inoculation experiments give proof of this relationship, although Stakman and Levine[3] found *B. fendleri* rusted with *P. graminis* at Highland Park, Rochester, New York. Mains[4] indicated some discrepancies in the relation of *B. fendleri* to *P. montanensis* which suggest a confusion with *P. graminis.*

As *B. fendleri* is prevalent in sections of Colorado not far removed from cultivated areas, it seemed worth while to test its susceptibility to *P. graminis tritici* under controlled conditions. This led to the inoculation of young, rapidly growing shoots of *B. fendleri* with sporidia of *P. graminis tritici.* These sporidia were obtained from germinating teliospores borne on *Hordeum jubatum* growing some 200 miles from any *B. fendleri.* The teliospores were germinated during the winter of 1924–25, and the sporidia sown on the *B. fendleri* shoots in the greenhouse. After inoculation, the plants were kept in a moist chamber for three days and then transferred to muslin-covered booths. Within seven days from the time of inoculation, profuse pycnial development was manifest, and in 21 days normal aecia developed in large numbers on the leaves. The infection was so extensive that many of the leaves were killed. The character of the aecia and the spore measurements are those of *P. graminis.* Parallel inoculations on *B. vulgaris* were equally successful.

Inoculations on Little Club wheat with the aeciospores produced profuse uredinial infection in eight days. The uredinia were large and confluent, and the spores were of the same size, shape, and pore arrangement as in *P. graminis.* No paraphyses were noted.

This test, successively repeated, definitely places *B. fendleri* among the susceptible species of the genus *Berberis,* and indicates beyond doubt that this shrub can act as an alternate host for *P. graminis tritici.*—L. W. DURRELL, Colorado Agricultural College, and E. A. LUNGREN, Office of Cereal Investigations, U. S. Dept. Agr.

Similarity of Bacterial Diseases of Avocado, Lilac, and Citrus in California.—During the spring of 1925, a new bacterial blemish on the fruit of avocado (Guatemalan type) and a bacterial disease of the lilac were studied. These new diseases are apparently caused by *Pseudomonas citriputeale,* which causes the Citrus black pit and blast. This organism was first described as causing a depressed spot on lemons.[5] Later, Citrus blast was

[3] Stakman, E. C., and M. N. Levine. A partial report on the susceptibility and resistance of *Berberis* and related genera to stem rust. Cereal Courier **15**: 278–287. 1923.

[4] Mains, E. B. The heteroecism of *Puccinia montanensis, P. koeleriae,* and *P. apocrypta.* Mycologia **13**: 315–322. 1921.

[5] Smith, C. O. Black pit of lemons. Phytopath. **3**: 277–283. 1913.

described and the causal organism isolated.[6] Finally the two troubles were again studied[7] and found to be two manifestations of the same organism. The spots on the fruit of avocado are brownish to black, firm, irregular to circular, 2–10 millimeters or larger upon coalescing. They do not extend far into the flesh but are limited to the rind. The lenticels are the channels of infection. The California lilac disease forms a circular spot 2–5 millimeters on the leaves, and a definite lesion on the stem. The causal organisms from both have been isolated, and their cultural characteristics and pathogenicity on different hosts are being studied, as well as their relationship to the European lilac disease caused by *Pseudomonas syringae*.[8] This investigation is well in progress, and the results thus far indicate that the Citrus blast, the avocado blemish, and the California lilac disease are closely related and probably are caused by the same organism.—CLAYTON O. SMITH, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

Holcus Bacterial Spot on Species of Holcus and Zea mays.—Bacterial leaf spots have been known on species of *Holcus* and *Zea mays* L. since Burrill's early, epoch-making work on bacterial diseases of plants. There has been, however, much confusion as to the identity of the causal agent or agents. A study of these bacterial spots has permitted a much clearer understanding of the cause and has definitely associated with the disease a single species of *Bacterium* which is quite cosmopolitan in its host range.

The symptoms of the Holcus bacterial spot vary somewhat on different hosts, a fact which doubtless has been responsible for much of the confusion in the earlier writings. However the lesions on all hosts are round, oblong, linear to irregular, and of varying size. On sorghum (*Holcus sorghum* L.), sudan grass (*H. sorghum* var. *sudanensis* (Piper) Hitchc.), and Johnson grass (*H. halepensis* L.), the spots are red or light centered with a red border, except on the variety of *H. sorghum* known as Shallu, on which the spots have a dark brown border. The lesions on pearl millet (*Pennisetum glaucum* (L.) R. Br.) are dark brown with a slight halo, while on corn (*Zea mays*) they are light to dark brown with a reddish brown border and a light green halo when viewed by transmitted light. The spots on foxtail (*Chaetochloa lutescens* (Weigel) Stuntz) are small and dark brown.

[6] Lee, H. A. A new bacterial disease of Citrus. Jour. Agr. Res. **9**: 1–8. 1917.

[7] Fawcett, H. S., W. T. Horne, and A. F. Camp. Citrus blast and black pit. Calif. Agr. Exp. Sta., Tech. Paper 5. 1923.

[8] Van Hall, C. J. J. De Seringenziekte, veroozaakt door *Pseudomonas syringae*. Bijdragen tot de kennis der bakterieele plantenziekten: 141–146. Amsterdam, 1902.

A white, fluorescent bacterium has been consistently isolated from the necrotic areas on the different hosts, and numerous cross-inoculations and cultural studies have shown that the organisms from the various hosts are identical. A careful comparison of the causal organism with previously described bacterial organisms on these hosts, as well as closely related hosts, indicates that this disease is caused by an undescribed *Bacterium*. The organism is briefly characterized as follows:

Bacterium holci n. sp.[9]

Short cylindrical rods, rounded at the ends, solitary or in pairs; average size .73μ by 2.13μ; motile by 1 to 4 polar flagella at one pole only; no spores, involution forms, or capsules; strictly aërobic; stains readily with gentian violet or carbol fuchsin; gram-negative.

Surface colonies on beef-peptone agar are round, raised, smooth, entire margin, finely amorphous; grayish white in reflected light, slightly greenish fluorescent in transmitted light.

Gelatin rapidly liquefied; nitrates reduced; no indol; no gas from various carbohydrates; acid from dextrose and saccharose; no acid in milk; casein digested without curdling; growth and greenish pigment formation in Uschinsky's and Fermi's solution; no growth in Cohn's solution; tolerates six per cent sodium chloride in pH 7 beef-peptone broth; grows in pH 5.0, but not in pH 4.5 beef-peptone broth.

Temperature growth range from 0° to 35° C., optimum between 25° and 30° C.; thermal death point, 49° C.; resistant to freezing in water; susceptible to desiccation on glass, but resistant on *Holcus sorghum* seed. Killed by 45-minute exposure to direct sunlight.

Group number 211.2323133.

Pathogenic on *Holcus sorghum* L., *H. sorghum* var. *sudanensis*, (Piper) Hitchc., *H. halepensis* L., *Pennisetum glaucum* (L.) R. Br., *Zea mays* L., *Z. mays* var. *saccharata* Bailey, and *Chaetochloa lutescens* (Weigel) Stuntz.—JAMES B. KENDRICK, Plant Pathological Laboratories, Iowa State College, Ames, Iowa.

The 1925 Summer Field Meeting—Cereal Disease Conference. The 1925 cereal disease field meets and conferences took place this year in three different States, Iowa, Minnesota, and North Dakota, starting July 8 at Ames and closing July 14 at Fargo. The meeting was authorized and held under the direction of the Advisory Board and under the immediate supervision of a general committee. Local committees for each state arranged

[9] According to Migula's classification and the revision adopted by the committee of the Society of American Bacteriologists, the combination would be *Pseudomonas holci* n. sp. Winslow, C.-E. A. et al. The families and genera of the bacteria. Jour. Bact. 5: 191–229. 1920.

programs to provide opportunity for a study of conditions and methods of disease control in operation for each of the major crops, including garden vegetables and truck crops.

The total attendance registered at the three meetings was 105. The registration at Ames was 42, at St. Paul 74, at Fargo 43. Of those in attendance at Ames, 30 went on to Minnesota. Of those at the Minnesota conference, 25 continued through to North Dakota. Twenty attended the meetings and field work in each of the three States, including representatives from the Department of Agriculture, Washington, D. C., from Ohio, Indiana, Wisconsin, Minnesota, Iowa, Kansas, New York, Delaware, North Dakota, Montana, New Mexico, and Hungary.

The rust and smut problems naturally attracted the greater number of workers. The barberry eradication forces were well represented by Dr. F. E. Kempton and the state leaders from Indiana, Ohio, Minnesota, Montana, Wisconsin, Iowa, and North Dakota. Within each of the states this number was increased by the attendance of various district workers. The Divisions of Agronomy at each of the institutions cooperated very closely with the pathologists and were particularly helpful in explaining to the visiting delegates the various field experiments under way. At Ames a full day was given to the study of the work in the laboratories, greenhouses, and the field plots on the separate farms. The first half-day at St. Paul and at Fargo was given over to a study of the experimental plots, and the remaining day and one-half to trips into the surrounding farming country. At Ames, attention was given to experiments on seed treatment of sweet corn, to studies on crown gall, the cereal disease gardens, plots for breeding oats for resistance to crown rust, treatment methods with smuts, studies in cedar apple rust, cabbage yellows, potato seed treatment, field corn seed treatments, fertilizer experiments, and numerous studies in the vegetable gardens. At St. Paul the field and agronomy plots on the University Farm were inspected, including the disease control experiments and plant breeding nurseries. At Fargo the seed standardization gardens for potatoes, alfalfa, wheat and flax, the flax and wheat disease control gardens, agronomy plots in cereal breeding, crop rotation areas, and fertilizer areas were studied.

At each of the institutions the work was started by an address of welcome from the President. At Ames, Drs. Humphrey and Stakman gave very interesting outlines on the high lights of plant disease control as accomplished during the past two decades. At Minnesota, all speech making was eliminated from the evening program. The lady members of the Division of Plant Pathology put on an interesting tableau-like pageant, "The Dance of the Rust Spores." Through posing, singing, and dancing, the young women, aided by an artful arrangement of specially painted scenery repre-

senting the wheat crop and the barberry bushes, and through skillful manipulation of properly colored toy balloons, portrayed the life history of the black stem rust. This was followed by a moving picture outlining the details of the life history of the rust plant in a splendid educational way. At Fargo at the evening dinner a number of speakers were called upon to outline the status of their chief investigations. Each of the evening meetings was a splendid exhibition of the proper mixing of pleasure with the real purposes of cooperative education. At different times, sometimes in the field, sometimes on the lawns at noon, splendid expositions of the present status of their investigations were outlined by Drs. Dietz, Mains, Tisdale, Levine, Husz, and others. At the closing session at Amenia, North Dakota, Dr. F. E. Kempton gave an interesting survey of the present status of barberry eradication in the thirteen States in which the work is now being pushed.

These cereal field meets and conferences have very greatly aided in bringing together the investigators and emphasizing the importance of cooperative effort in the different States. An outstanding feature of this meeting was the effort on the part of each investigator to make clear the work as laid out in his experiments and as shown under the local conditions, so that the visitors working on similar problems could not fail to gain much valuable information. The field tours into the farming and land development districts of the three States also furnished splendid opportunity to see wherein the results of past investigations were being put into operation. There were places in each of the States wherein it was evident that no attention whatever had been given to such matters by the average grower, and there were other communities in which it was apparent that the work done by plant pathologists in the past decades had taken effect in practical agriculture. This was particularly marked in the work of the larger land development projects, under the management of skilled or trained foremen.

In each State over 200 miles of travel was made through the very best cereal districts. The workers had opportunity to get into the field at any point desired in addition to those outlined in the local programs. These drives gave opportunity to notice the astonishing effect of the present barberry eradication work in Iowa, Minnesota and North Dakota. It was clear that something had been done to stem rust of oats, wheat and barley. Great districts were found in each of the three States in which it was difficult for the experts to locate anything like destructive forms of infection. There were other areas visited in which the rust infections were astonishingly severe, quite discouraging to those who had expected too early results from the eradication accomplished to date.

Another very outstanding feature is the splendid line of investigations looking toward the establishment of accurate fundamental methods of breeding for disease control. The accomplishments in each of the States, particularly on the experimental grounds, must certainly have been very enlightening to the best informed workers in the group. No one of these States but has a number of highly important cereals now in commercial use that have been bred for disease resistance in such manner as not only to result in practical control of the specific disease but clearly proved to be better yielders and of better quality than the varieties previously in use. From a study of the breeding plots at these three institutions, it must be clear to any intelligent investigator that there is probably no type of cereal plant which cannot be very much improved by breeding and selecting processes now in use; and that by intelligent action one can very greatly hasten the coming into use of types which are far more suited to local conditions than those now commonly sown.—H. L. BOLLEY, for the General Committee, Agricultural College, Fargo, North Dakota.